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Baseline Studies of Fish Blood

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16. ABSTRACT

The objectives of this work were to develop hematological methodologies for fish, determine effects of stocking density on fish hematology, define, characterize, quantify baseline hematological values of adult bluegill, and provide a selected and annotated bibliography of baseline and temperature studies on fish blood.

A survey of the literature indicated that more studies are needed to determine normal (nonstressed) blood parameter values for freshwater teleosts. Standard clinical methods, with some modifications for small blood samples, are adequate for fish hematological studies. A density stress syndrome seems to exist for goldfish, catfish, and bluegill. Blood dyscrasias associated with density stress include smaller and rounder red blood cells, hyperglycemis, an increase in albumin/protein ratios, and decreased values for hematocrit and hemoglobin. Variations in hematology were observed in adult bluegill depending on the culture complexity. Results indicated that monoculture data were valid to use as controls as well as for use in bioassays to characterize potential environmental perturbations. Baseline hematological values generated for adult bluegill showed that hematological relationships depended on sex and season. Good hematological indicators appear to be those associated with red blood cell morphology and erythrocyte-thrombocyte distribution.

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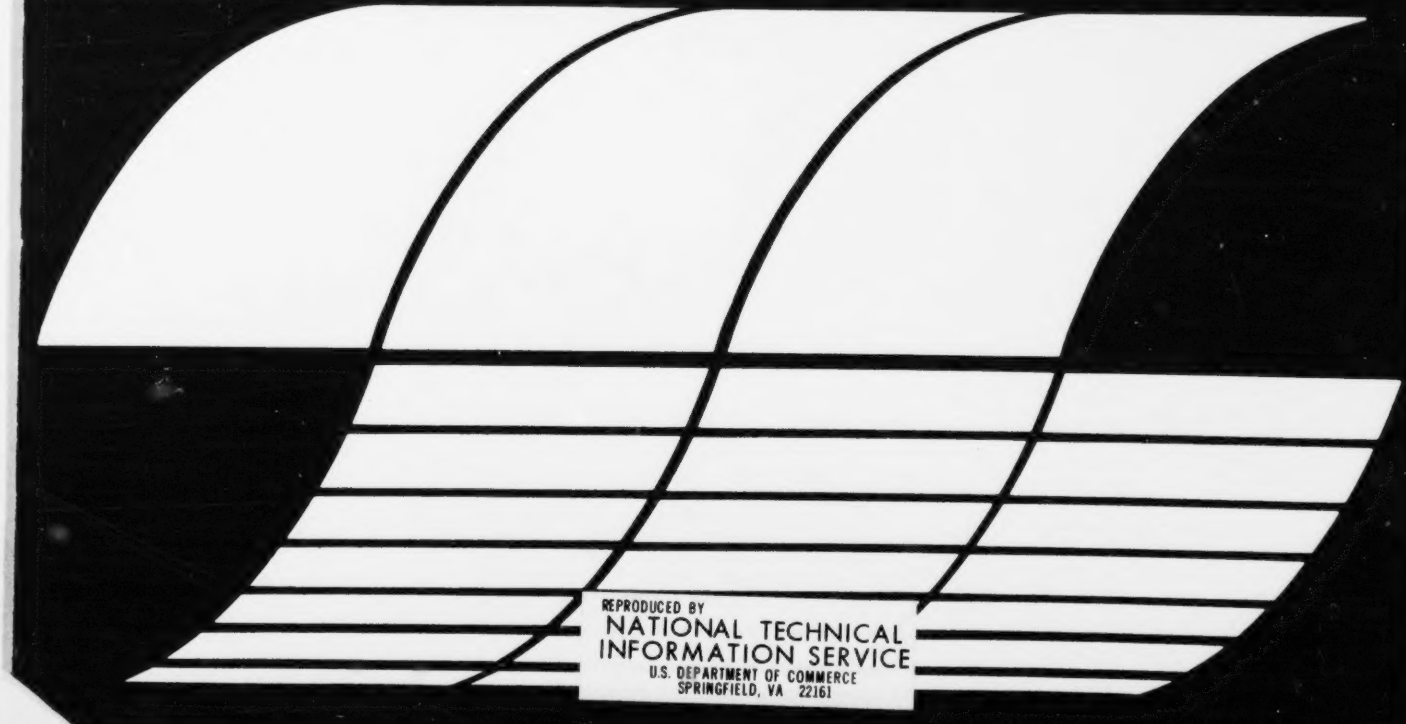

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BASELINE STUDIES OF FISH BLOOD

by

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ABSTRACT

Recent literature of baseline studies on fish blood chemistry, physiology, blood cell abundance, and morphology are reviewed. Studies on possible sources of interspecific and intraspecific variation in baseline values of fish blood are discussed. A selected and annotated bibliography of baseline and temperature studies on fish blood is included in this report.

Hematological methodologies for fish were developed and implemented in a study to determine the effects of stocking density on blood chemistry and cell morphology. Adult goldfish were held at two loading densities: 0.80 and 4.19 fish centimeters per liter of water. Consistent decreases of hematocrit and hemoglobin concentrations, with an increase in erythrocyte sedimentation rate, were found to be related to density. Density effects on blood chemistry were apparently masked by fish size and sex. Morphological changes associated with fish held at high density included decreased counts of red blood cells and thrombocytes and an increase in lymphocytes and total leucocytes. Smaller red blood cells were also found in fish held at high density.

Intensive culturing of catfish held at 0.18 kg/m^3, 224 kg/m^3, 337 kg/m^3, and 433 kg/m^3 resulted in changes in red blood cell morphology. Smaller and rounder red blood cells were consistently found in intensive fish cultures. It was also determined in this study that 200 cells need to be measured to adequately characterize cell features.

The effects of culture composition on the hematology of adult bluegill were examined to determine the validity of using monocultures to characterize potential environmental perturbations. Results indicated that monoculture data were valid to use as controls. Hematological responses of bluegill associated with differences in stocking density of a second fish species were similar to hematological responses of goldfish and catfish stocked at different densities. A density stress syndrome was confirmed at the cellular level and identified for the chemical level. More complex fish cultures, comprised of three fish species, were studied. Blood dyscrasias were also identified for triculture conditions.

Baseline hematological values generated for adult bluegill showed that hematological relationships depended on sex and season.

CONTENTS

Page

Abstract iii

List of Tables ix

Acknowledgements xii

Section 1. Introduction 1

I. Objectives 1

II. Literature Review on Baseline Studies of Fish Blood 1

A. Osmotic Concentrations 2
B. Glucose Concentrations 2
C. Total Protein and Protein Component Concentrations 2
D. Hemoglobin Concentrations 3
E. Hematocrit Levels 3
F. Erythrocyte Sedimentation Rates 4
G. Hematological Indices 4
H. Mean Erythrocyte Volume 4
I. Mean Erythrocyte Hemoglobin 4
J. Mean Erythrocytic Hemoglobin Concentration 5
K. Erythrocyte Abundance 5
L. Erythrocyte Morphology. 6
M. Leucocyte-Thrombocyte Abundance 6
N. Cellular Distribution 7
O. Summary and Conclusions 8

III. Potential Sources of Variation in Fish Blood. 8

A. Osmotic Concentrations 9

1. Capture, transport and handling . . 9
2. Crowding. 9
3. Season 9
4. Temperature 9
5. Sex and Size 10

CONTENTS (Continued)

Page

B. Glucose Concentrations 10

1. Handling and Anesthesia 10
2. Diet 10
3. Crowding 11
4. Season 11
5. Temperature 11
6. Strain 12
7. Sex 12

C. Protein Concentrations. 12

1. Capture and Transport 12
2. Anesthesia 12
3. Diet 12
4. Season 12
5. Temperature 13
6. Age and Sex 13

D. Hemoglobin Concentration 13

1. Capture, Transport, Handling and Anesthesia 13
2. Diet 14
3. Crowding. 14
4. Season 14
5. Temperature 14
6. Strain 16
7. Age 16
8. Sex 16

E. Hematocrit Levels 16

1. Capture, Transport, Handling, and Anesthesia 16
2. Diet 17
3. Crowding 17
4. Season 17
5. Temperature 17
6. Strain 19
7. Age or Size 20
8. Sex 20

F. Hematological Indices 20

1. Crowding 21
2. Season 21
3. Temperature 21
4. Size and sex 22

CONTENTS (Continued)

Page

G. Erythrocyte Sedimentation Rates 23

1. Diet and Crowding 23
2. Temperatures 23
3. Strain 23
4. Age, Size, and Sex 23

H. Erythrocyte Abundance 23

1. Capture and Transport 24
2. Crowding and Diet 24
3. Season 24
4. Temperature 24
5. Strain 25
6. Age and Size 25
7. Sex 26

I. Erythrocyte Morphology 26

1. Crowding 26
2. Age, Size, and Sex 26

J. Leucocyte-Thrombocyte Abundance 26

1. Capture and Transport 26
2. Crowding 27
3. Season 27
4. Age or Size 27
5. Sex 27

K. Cellular Distribution 27

1. Anesthesia 27
2. Season 27
3. Age and Sex 27

L. Summary and Conclusions 28

Section 2. Conclusions 29

Section 3. Recommendations 30

Section 4. Experimental Procedures 31

I. Fish Sources 31

II. Blood Sample Collection 31

CONTENTS (Continued)

Page

III. Hematological Techniques 31

- A. Hematocrit 31
- B. Erythrocyte Sedimentation Rates 31
- C. Hemoglobin 32
- D. Blood Cell Enumeration 32
- E. Osmolality 32
- F. Glucose Concentrations 32
- G. Total Protein Concentration 33
- H. Albumin 33
- I. Percentage of Albumin to Total Protein 33
- J. Differential Counts 33
- K. Cell Morphology 34

IV. Goldfish Density Study. 34

V. Catfish Density Study 34

VI. Culture Complexity Study 35

VII. Bluegill Hematology 36

Section 5. Results and Discussion. 37

I. Goldfish Studies 37

- A. Blood Chemistry 37
- B. Cell Morphology and Distribution . . . 39

II. Catfish Studies 42

III. Culture Complexity Studies 44

IV. Bluegill Hematology 46

- A. Blood Chemistry 46
  - 1. Hemoglobin 46
  - 2. Hematocrit 46
  - 3. Erythrocyte Sedimentation Rate . . 46
  - 4. Protein 46
- B. Hematological Indices 46
  - 1. Erythrocyte Abundance 47
  - 2. Mean Erythrocytic Volume. 47
  - 3. Mean Erythrocytic Hemoglobin . . . 47
  - 4. Mean Erythrocytic Hemoglobin Concentration 47

CONTENTS (Continued)

Page

C. Leucocyte-Thrombocyte Differentials . . 47

1. Thrombocytes 47
2. Lymphocytes 47
3. Neutrophils 47
4. Eosinophils 47
5. Basophils 47
6. Hemoblasts. 47

D. Red Blood Cell Morphology 48

1. Cell Length 48
2. Cell Width 48
3. Cell Height 48
4. Cell Roundness 48
5. Cell Area 48

E. Hematological Relationships 48

F. Sex-Seasonal Influences 49
G. Baseline Results 49

Section 6. A Selected and Annotated Bibliography of Baseline and Temperature Studies on Fish Blood 86

TABLES

Number Page

1 Blood Chemistry of Some Freshwater Teleosts 51

2 Blood Physiology of Some Freshwater Teleosts 52

3 Erythrocyte Morphology of Some Freshwater Teleosts . . . 53

4 Cellular Distribution in the Blood of Some Freshwater Teleosts 54

5 Hematological Values for Goldfish, *Carassius auratus* L., at Two Different Densities 55

6 Summary of Results for Correlation Coefficient Between Length and Selected Blood Characteristics 56

7 Comparisons of Selected Blood Characteristics Between Sexes for Combined and Individual Density Groups . . . 57

8 Comparisons of Selected Blood Characteristics Between Two Loading Densities for Each Sex 58

9 Number of Cells Per Cubic Millimeter in *Carassius auratus* L., at Two Loading Densities 59

10 Summary of the Results of the Student's T-Test Between Treatment Groups for Each Cell Type Examined in Leucocyte-Thrombocyte Differential Counts 60

11 Summary of Analysis of Variance Tests for Blood Cell Morphology of *Carassius auratus* L., at Two Loading Densities with Two Hundred Cell Measurements for Each of the 111 fish 61

12 Blood Cell Morphology Statistics of *C. auratus* L., at Two Holding Densities 62

13 Calculated Values for Area and Roundness of Red Blood Cells 63

14 Effects of Loading Density on Cell Morphology of Channel Catfish 64

15 Comparison of Cell Morphology for Group 1 (50 Cell Measurements Per Fish) and Group 2 (250 Cell Measurements Per Fish) 65

TABLES (Continued)

Number		Page
16	Composition of Fish Cultures	66
17	Effects of Culture Composition on Bluegill Hematology. .	67
18	MANOVA Test Criteria for the Hypothesis of No Overall Effect of Group. Excludes Data for Plasmaosmolality, Glucose, Albumin, Protein, and Alb/P.	68
19	Results of Univariate Analysis of Variance	69
20	Percentage of the Total Variation of the Hematological Variables for Culture Stress	70
21	Results of Partial Correlations for Culture Stress . . .	71
22	Percentage of the Total Variation of the Hematological Variables for the Control Groups (Groups 1 and 2) . .	72
23	Results of Partial Correlations for the Control Groups (Groups 1 and 2)	73
24	Percentage of the Total Variation of the Hematological Variables for Density Stress of Second Species	74
25	Results of Partial Correlation Analysis for Density Stress	75
26	Percentage of the Total Variation of the Hematological Variables for a Complex Culture Consisting of Three Fish Species	76
27	Results of Partial Correlation Analysis for Triculture	77
28	Summary of Fish Size and Blood Chemistry of Bluegill . .	78
29	Summary of Hematological Indices of Bluegill	79
30	Summary of Leucocyte-Thrombocyte Distribution (%) of Bluegill Blood	80
31	Summary of Red Blood Cell Morphology of Bluegill	81
32	Results of Partial Correlation Analysis	82
33	MANOVA Test Criteria for the Hypothesis of No Overall Effect for Sex, Season, and Sex-Season Interaction . .	83

TABLES (Continued)

Number | Page

34 Percentage of the Total Variation of the Hematological Variables 84

35 Some Blood Parameters of Bluegill 85

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SECTION 1

INTRODUCTION

Contemporary studies indicate that fish blood shows promise as a biomonitoring index for assessing water quality (Block 1974, MacLeay 1975, Wedemeyer 1976). Since fish blood is sensitive to a variety of environmental conditions, data are needed to determine which blood parameters might be good indicators of stress. Basic information, such as seasonal and sexual influences, culturing methods and loading densities, needs to be known before variations from environmental perturbations can be identified. It is also important to examine previous studies to determine the status of knowledge in this area.

I. Objectives

The objectives of this report are manifold. One of the objectives of this report is to review the previous literature on baseline studies of fish blood and examine potential sources of variation in these blood parameters. The scope of this review will be limited to the parameters studied in our experiments, namely osmotic concentration of the plasma (or serum), blood glucose, blood protein, hemoglobin and hematocrit levels, hematological indices, erythrocyte sedimentation rate, erythrocyte abundance and morphology, and leucocyte-thrombocyte abundance.

A second objective of this work is to develop and implement hematological methodologies for fish. A concomitant objective was to determine whether hematological characteristics of goldfish are influenced by density under experimental conditions.

A third objective is to examine physiological effects of intensive culturing on catfish hematology. A concomitant objective of this study was to resolve sample sizes for cell morphology measurements.

A fourth objective is to examine the effects of culture composition on the hematology of adult bluegill. The emphasis of this study was to determine (1) the validity of using monospecific cultures in hematological bioassays, (2) effects of stocking density of a second species while that of the bluegill was held constant, and (3) effects of a more complex culture, comprised of three-fish species, on bluegill hematology.

A fifth objective, and main objective, is to define, characterize and quantify baseline hematological values of adult bluegill.

A sixth objective is to provide a selected and annotated bibliography of baseline and temperature studies on fish blood.

II. Literature Review on Baseline Studies of Fish Blood.

The recent literature of baseline studies on fish blood is reviewed in this section. Many studies have been performed in an attempt to establish "normal" or nonstressed values for fish blood chemistry (osmotic, total protein and protein component, glucose, and hemoglobin concentrations), blood physiology (hematocrit, erythrocyte sedimentation rate, hematological indices, erythrocyte counts, and leucocyte-thrombocyte counts), erythrocyte morphology and blood cellular distribution. However, the information for any one blood parameter is limited, as is the information for any one species of freshwater teleosts. Therefore, although interspecific (between species) and intraspecific (within species) differences will be discussed for many blood parameters, no firm conclusions regarding the extent of these differences can be established.

A. Osomotic Concentrations. Some baseline values for freshwater fish are given in Table 1. As can be seen from this table, except for euryhaline species, the osmotic concentrations are between 200 to 300 mosm/ℓ. Values for the euryhaline species, banded killifish and mummichogs, are somewhat higher (Ahokas and Sorg 1977, Umminger 1970b). No data were found in interspecific and intraspecific variations of plasma osmotic concentrations.

B. Glucose Concentrations. The baseline glucose levels of freshwater teleosts are given in Table 1. The glucose levels appear to vary over a relatively broad range, viz. 42 mg/100 ml. in goldfish (Catlett and Millich 1976) to 202 mg/100 ml. in rainbow trout (Barnhart 1969).

No studies were found on the interspecific and intraspecific variations of glucose levels. However, the values for rainbow trout suggest that there may be some intraspecific variation in glucose levels.

C. Total Protein and Protein Component Concentrations. The normal total protein concentrations of freshwater teleosts usually fall between 1.9 and 8.0 g/100 ml. (Table 1). However, the baseline total protein levels of mudfish were considerably higher than for other fish reported here. The protein levels of salmonids and cyprinids appear to vary over a fairly broad range. In addition, the protein levels of any one species may be somewhat variable, as indicated by rainbow trout and goldfish.

The albumin levels of some fish are listed in Table 1. The albumin level of brook trout (2.1 g percent) and carp (2.8g percent) were similar, representing 60-68 percent of the total protein present (Field et al. 1943). Albumin values were also recorded in the blood of smallmouth bass and rainbow trout, representing 37-61 percent and 41-48 percent of the total protein, respectively (Shell 1961, Meisner and Hickman 1962). From this limited information it appears that the proportion of albumin to total protein varies between species of teleosts. There was no information found on intraspecific variation in total protein and protein components.

D. Hemoglobin Concentrations. In contrast with the other blood chemistry parameters, many studies have been done to determine normal hemoglobin levels in several species of teleosts (Table 1). Baseline hemoglobin levels for freshwater teleosts range from 6.6g/100 ml. in channel catfish (Haws and Goodnight 1962) to 10.6 g/100 ml. in mountain whitefish (McKnight 1966) and goldfish (Burton and Murray 1979). The majority of information on normal hemoglobin levels were found for salmonids and cyprinids, both of which appeared to vary between 7-11 g/100 ml.

Some interspecific comparisons have been performed on normal hemoglobin levels of teleosts. Field et al. (1943) found that brook trout had higher hemoglobin levels than carp. However, interspecific comparisons between bigmouth buffalo and smallmouth buffalo, and between brown bullhead and channel catfish revealed no significant differences in hemoglobin levels (Chlebeck and Phillips 1969, Haws and Goodnight 1962).

No studies were found on intraspecific variation in hemoglobin levels of teleosts. From Table 1 there appears to be some intraspecific variation in baseline values of rainbow trout and goldfish, whereas there does not appear to be much variation in baseline values of golden shiners.

E. Hematocrit Levels. Many studies have been performed to determine baseline hematocrit (Hc) values for a wide variety of fish (Table 2). The baseline hematocrit values for freshwater teleosts vary between 23 percent and 57 percent. The majority of information found on baseline hematocrit values were reported for salmonids and cyprinids, which ranged from 23-55 percent and 29-42 percent, respectively.

Some interspecific comparisons were performed on hematocrit levels of some fish. Production brown trout had significantly lower hematocrit values than production brook trout and rainbow trout (Snieszko 1961). However, no significant differences in hematocrit values were found between brook trout and carp (Field et al. 1943), between brown bullhead and channel catfish (Haws and Goodnight 1962), and between smallmouth buffalo and bigmouth buffalo (Chlebeck and Phillips 1969).

No studies were found on the intraspecific differences in hematocrit levels of teleosts. However, some comparisons can be made from the data reported here. There appears to be considerable intraspecific variation in the hematocrit levels of brook trout, rainbow trout, and goldfish. The hematocrit values of channel catfish, golden shiners, and brown trout, however, showed little variation.

F. Erythrocyte Sedimentation Rates (ESR). Normal erythrocyte sedimentation rates for some fish are given in Table 2. The baseline ESR values appear to vary from 1.2 mm/hr (mountain whitefish and brown trout) to 2.0 mm/hr in goldfish (McKnight 1966, Blaxhall and Daisley 1973, Burton and Murray 1979). The majority of information found on ESR was limited to salmonids. These values range from 1.2 mm/hr to 1.9 mm/hr.

No studies were found on intraspecific variation in ESR values of teleosts. The only comparison which can be made from Table 2 indicates that there may be little intraspecific variation in ESR values, as in the case of rainbow trout (McCarthy et al. 1973, Barnhart 1969).

G. Hematological Indices. The hematological indices include the mean erythrocytic volume (MEV), mean erythrocytic hemoglobin (MEH) and mean erythrocytic hemoglobin concentration (MEHc). The baseline values for each parameter are discussed below.

H. Mean Erythrocytic Volume (MEV). The reported values (Table 2) for the mean erythrocytic volume varied over a wide range, viz 165 m^3 in goldfish (Falkner and Houston 1966) to 380 m^3 in rainbow trout (McCarthy et al. 1973). These values were reported for salmonids and cyprinids only.

No studies were found on the interspecific and intraspecific variations in mean erythrocytic volume. However, an intraspecific comparison was made between the goldfish values listed in Table 2. These values indicate that there may be considerable variation in the mean erythrocytic volume of goldfish.

I. Mean Erythrocytic Hemoglobin (MEH). Normal values for the mean erythrocytic hemoglobin (Table 2) ranged from 29 mmg in bluegill to 84 mmg in spotted sucker (Smith et al. 1952). The majority of studies were performed on cyprinids and catostomids, both of which showed considerable variation in mean erythrocytic hemoglobin values. Less variation was found in salmonids and ictalurids, each of which had only two reported values listed here.

Some comparative studies were performed to assess interspecific differences in mean erythrocytic hemoglobin values. Smith et al. (1952) found some significant differences between the mean erythrocytic hemoglobin values of smallmouth buffalo, goldfish, carp, yellow bullhead, black bullhead, spotted sucker, bluegill, warmouth, and white crappie. However, similar mean erythrocytic hemoglobin values were found between smallmouth buffalo and bigmouth buffalo (Chlebeck and Phillips 1969) and between brook trout and carp (Field et al. 1943).

No studies were found on intraspecific comparison of mean erythrocytic hemoglobin levels. From the available data in Table 2, however, the intraspecific variation in baseline values of carp, goldfish, and smallmouth buffalo is between 8 and 22 mmg.

J. Mean Erythrocytic Hemoglobin Concentration (MEHc). The baseline values for mean erythrocytic hemoglobin concentration ranged from 19 percent in rainbow trout (McCarthy et al. 1973) to 61 percent in spotted sucker (Smith et al. 1952). Most of the information was found on cyprinids and centrarchids, which ranged from 26-42 percent and 38-49 percent, respectively.

Comparative studies were performed between the baseline mean erythrocytic hemoglobin concentrations of some freshwater teleosts. Smith et al. (1952) found interspecific differences between the values of carp, goldfish, smallmouth buffalo, spotted sucker, black bullhead, yellow bullhead, bluegill, warmouth, and white crappie.

No studies were found on the intraspecific variations in mean erythrocytic hemoglobin concentrations of teleosts held under baseline conditions. From the information listed in Table 2, however, the reported values for goldfish can be compared. It appears that there is little variation in the reported values for mean erythrocytic hemoglobin concentration, which were between 26 and 30 percent (Smith et al. 1952, Burton and Murray 1979). The reported values for mean erythrocytic iron concentration was 17 percent (Falkner and Houston 1966).

K. Erythrocyte Abundance. Several studies have been performed to determine the normal erythrocyte abundance in the blood of a variety of species of teleosts (Table 2). The normal erythrocyte count varied from 800,000 cells/mm^3 in lake trout (Piper and Stephens 1962) to 2,600,000 cells/mm^3 in bluegill (Smith et al. 1952). The majority of these studies were performed on salmonids and cyprinids. Baseline values for salmonids ranged between 800,000 cells/mm^3 in rainbow trout (Piper and Stephens 1962) and 1,570,000 cells/mm^3 in mountain whitefish (McKnight 1966). Normal erythrocyte counts in cyprinids fell between 840,000 cells/mm^3 in carp (Field et al. 1943) and 2,150,000 cells/mm^3 in goldfish (Catlett and Millich 1976). Considerable variations were also apparent in ictalurids and centrarchids.

Comparative studies were found on the erythrocyte counts between some species of teleosts. Significant interspecific variations were found between the erythrocyte counts of eight species of warmwater teleosts, with spotted suckers having the lowest erythrocyte counts and bluegill having the highest erythrocyte counts of all species examined (Smith et al. 1952). In addition, significantly higher erythrocyte counts were found in smallmouth buffalo (Chlebeck and Phillips). Channel catfish also had higher baseline erythrocyte counts than brown bullhead (Haws and Goodnight 1962).

No studies were found on intraspecific variations in erythrocyte counts; however, some information can be gained from the data in Table 2.

There appears to be considerable variation in the erythrocyte counts of rainbow trout, carp and goldfish. The reported erythrocyte counts of smallmouth buffalo, however, are much closer in agreement.

L. Erythrocyte Morphology. Baseline values for the length, width, roundness, and area of erythrocytes from the blood of some freshwater teleosts are listed in Table 3. The normal erythrocyte length varied from 10.9 μm in bluegill (Smith et al. 1952) to 17.8 μm in lake trout (Lieb et al. 1953). The normal erythrocyte width ranged from 6.4m in bluegill (Smith et al.) to 10.4 μm in rainbow trout (McCarthy et al. 1973) and lake trout (Lieb et al. 1953). The baseline erythrocyte roundness values fell between 0.58 in largemouth bass and 0.85 in yellow bullhead (Smith et al. 1952).

Erythrocyte morphology was examined in a variety of teleosts (Table 3). Considerable variation in erythrocyte length was found in salmonids, whereas little variation was found in cyprinids, catostomids, ictalurids, and centrarchids. Erythrocyte width was more variable in cyprinids and ictalurids than in catostomids, salmonids, and centrarchids. Roundness varied more in salmonids, cyprinids, ictalurids, and centrarchids than in catostomids. Erythrocyte area was considerably variable in salmonids, cyprinids, and ictalurids, whereas it was less variable in catostomids and centrarchids.

Comparative studies were performed on the erythrocyte morphology between some species of freshwater teleosts. Although there was no significant change in the erythrocyte shape, significantly smaller erythrocytes were found in channel catfish than in brown bullhead (Haws and Goodnight 1962). American grayling also had significantly smaller erythrocytes than lake trout (Lieb et al. 1953). Interspecific differences in erythrocyte size and shape were also found in eight warm water species of teleosts (Smith et al. 1952). The longest erythrocytes were found in smallmouth buffalo and the smallest were found in bluegill. Erythrocyte width was greatest in European carp and was smallest in yellow bullhead. Oval erythrocytes were found in goldfish, smallmouth buffalo, and spotted sucker, whereas round erythrocytes were found in yellow bullhead and black bullhead. Erythrocytes were oblong and somewhat pointed in warmouth and largemouth bass (Smith et al. 1952).

No studies were found in intraspecific variation in erythrocyte morphology. The values in Table 3 for goldfish, however, can be compared. These values indicate that there is little variation in erythrocyte length, width, roundness and area.

M. Leucocyte-Thrombocyte Abundance. The baseline values for leucocyte-thrombocyte counts of freshwater teleosts (Table 2) ranged from 3,700 cells/mm^3 (excluding thrombocytes) in carp (Field et al. 1943) to 73,600 cells/mm^3 in goldfish (Watson et al. 1963). The majority of studies on baseline leucocyte-thrombocyte counts were performed on salmonids and cyprinids. The leucocyte-thrombocyte counts ranged between 3,900 cells/mm^3 (excluding thrombocytes) in brook trout (Field et al. 1943) and 56,400 cells/mm^3 in coho salmon (McLeay 1975). Values for leucocyte-thrombocyte counts of cyprinids varied between 3,700 cells/mm^3 (excluding thrombocytes) in carp (Field et al. 1943) and 73,600 cells/mm^3 in goldfish (Watson et al. 1963).

A few comparative studies were found between the leucocyte-thrombocyte counts (or leucocyte counts) of different species of teleosts. Chlebeck and Phillips (1969) found that bigmouth buffalo and smallmouth buffalo had similar leucocyte-thrombocyte counts. In addition, similar leucocyte counts were found between carp and brook trout (Field et al. 1943).

No studies were found in intraspecific variations in leucocyte-thrombocyte abundance. The data in Table 2, however, indicate that there may be considerable variation in the leucocyte-thrombocyte counts of goldfish.

N. Cellular Distribution. The lack of standardized nomenclature for fish leucocyte and thrombocyte cell types has caused some problems in reviewing the literature. Because this report is limited to the scope of our studies, the nomenclature of Watson et al. (1963), which is used in our studies, will be used in this report. According to this classification, leucocytes include lymphocytes and granulocytes (neutrophils, eosinophils, and basophils). The remaining cell types include erythrocytes, thrombocytes, hemoblasts, and macrophytes. The cellular distributions are usually determined by cellular differentials, but are sometimes determined by volumetric counts. Erythrocytes are not included in these determinations.

The cellular distribution of the blood from some species of teleosts are given in Table 4. The percentage distribution of thrombocytes in these studies ranged from 1.7 percent in rainbow trout (Schreck et al. 1976) to 82.0 percent in goldfish (Murray and Burton 1979). In the studies in which thrombocytes were included in the cellular distribution leucocytes accounted for 18 percent in goldfish (Murray and Burton 1979), and approximately 98 percent in rainbow trout (Schreck et al. 1976, McCarthy et al. 1973). Thus, there apparently are wide ranges in the proportions of thrombocytes and leucocytes present. This may be due to actual differences between the species or it may be due to the problem of correctly distinguishing between thrombocytes and small lymphocytes.

The most abundant leucocyte encountered in the majority of species was the lymphocyte (Table 4). Lymphocytes accounted for 89-94 percent of the total leucocytes in salmonids, except for mountain whitefish in which they accounted for 34 percent (McKnight 1966). Lymphocytes comprised approximately 80-95 percent of the total leucocytes found in cyprinids (goldfish).

Granulocytes (neutrophils, eosinophils, and basophils) were the next most common leucocytes found in most species of teleosts. The proportion of granulocytes to total leucocytes ranged from 5 percent in goldfish (Murray and Burton 1979) to 66 percent in mountain whitefish (McKnight 1966). The other cell types encountered, namely hemoblasts and macrophages, did not constitute more than 0.3 percent of the cellular distribution in any species of fish reported here.

Although no comparative studies were found on interspecific or intraspecific variations in cellular distributions, some of the values in Table 4 can be compared. The baseline cellular distribution of rainbow trout show little variation. However, there is considerable intraspecific variation in the proportion of lymphocytes (ranging from 80-95 percent) and granulocytes (ranging from 5-20 percent) of goldfish.

O. Summary and Conclusions. From the above information it is abundantly clear that more studies are needed to determine normal (nonstressed) blood parameter values for freshwater teleosts. There is a general lack of information on any one blood parameter for freshwater teleosts. In addition, very little work has been done on the blood picture of any one species of fish. For this reason, no baseline levels can be firmly established for any one species of teleosts.

The available information also indicates that there may be interspecific and intraspecific variations in the reported values. More information is also needed on the potential sources of these variations, so that these factors may be taken into account when trying to establish baseline (nonstressed) blood values.

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III. Potential Sources of Variation in Fish Blood

As can be seen from the previous section, many studies have been performed in an attempt to establish "normal" or baseline values for fish blood chemistry, physiology, and cellular morphology. However, variations in blood parameter values have been found between different teleostean species (interspecific differences) and within individual teleostean species (intraspecific differences).

Because these studies have been performed under a variety of environmental conditions, these variations may be the result of different environmental conditions and/or the result of differences inherent to the fish studied. For this reason, the effects of some of these sources of variation (handling, transport and capture, anesthesia, diet, crowding, season, temperature, population, age, size, and sex) are reviewed in this section.

A. Osmotic Concentrations. Potential sources of variation in the osmotic concentration of the plasma were investigated. These potential sources included capture, transport and handling, crowding, season, temperature, sex, and size.

1. Capture, transport, and handling. Capture and transportation stress may affect the plasma osmolarity values of teleosts. Freshwater mudfish had reduced osmolarity values within the first 2 to 3 days after capture and transportation. These values returned to normal within 7 to 10 days after the fish were transported to the laboratory (Hattingh and Van Pletzen 1974).

2. Crowding. Another environmental condition which may affect plasma osmolarity is fish loading density or crowding. In the only study found by the author on this factor, differences in the loading density did not appear to affect plasma osmolarity values of goldfish (Burton and Murray 1979).

3. Season. Seasonal intraspecific variations may occur in the fish plasma or sera. Gordon (1959) studied seasonal variations in the freezing point depression of serum in freshwater brown trout. The freezing point depressions of the sera of brown trout sampled in June to August, which was prior to the onset of the breeding season, were lower than those of trout sampled in September to April. There were no apparent seasonal changes during September to April (Gordon 1959).

4. Temperature. Several studies were performed to determine the effect of elevated temperatures on the osmotic concentrations of the plasma or serum in freshwater teleosts. The osmotic concentrations of the sera were similar in brown trout held at 10° and 20°C (Gordon 1959). In addition, similar osmolarity values were found between goldfish held in tapwater at temperatures of 10°, 20°, and 30°C. However, the plasma osmolarity values of goldfish held in demineralized water appeared to increase with temperature.

On the other hand, the plasma osmotic concentration was greater in rainbow trout held at 5°C than in trout held at 15°C (Rao 1969). Plasma osmolarity values also appeared to decrease at elevated temperatures in fall carp held at 33°C (Houston and Madden 1968, Houston et al. 1970), and in winter carp held at 7° and 27°C (Houston et al. 1970).

Cold acclimation and cold shock have been studied in some species of teleosts. No significant differences were found in plasma osmotic concentrations of summer carp held at 2° and 7°C (Houston et al. 1970). However, acclimation of goldfish and killifish to cold temperatures resulted in decreased plasma (or serum) osmolarity values (Catlett and Millich 1976, Umminger 1970b, 1971).

On the other hand, plasma osmolarity values of banded killifish increased during cold exposure (Ahokas and Sorg 1977). In addition, when 30°C channel catfish acclimated to 30°C, were subjected to an abrupt transfer to 10°C, there was a slight increase in plasma osmolarity values at 10 minutes post shock, followed by a large decrease in plasma osmolarity values. When the catfish were returned to 30°C, plasma osmolarity values remained depressed at 24 hours but returned to normal later (Block 1974).

5. Sex and size. The effects of fish sex or size on plasma osmolarity values have been investigated in some teleosts. A study on brown trout revealed that there were no significant variations in plasma osmolarity values between male and female brown trout (Gordon 1959). In addition, no significant correlations were found between fish length and the plasma osmolarity values of goldfish (Burton and Murray 1979).

B. Glucose Concentrations. Differences in blood glucose concentrations may be associated with the population of fish used in the study and/or with environmental or experimental conditions. Some of the potential sources of variation are discussed below.

1. Handling and anesthesia. Handling or anesthesia may be associated with changes in blood glucose levels. Handling of brook trout resulted in elevated blood glucose levels above those of anesthetized and control trout (Houston et al. 1971). In addition, electroshocked rainbow trout had elevated plasma glucose levels within 3 hours post-shock. These values remained elevated 6 hours after electroshocking (Shreck et al. 1976).

2. Diet. The diet of fish may also cause variations in blood glucose levels. There were no signficant differences in blood glucose levels of brook trout during temporary starvation (Phillips 1961). On the other hand, brook trout fed on a meat diet had lower blood glucose levels than control fish, while trout fed on a meat-meal diet had higher blood glucose levels than control trout (Phillips 1961). However, comparison of the blood glucose levels of rainbow trout fed on Santa Monica and Colorado diets, which differ mainly in the source of vegetable protein, revealed that diet had no significant effect on blood glucose levels (Barnhart 1969).

3. Crowding. Loading density may also alter blood glucose levels. When juvenile coho salmon were transferred from 8.1 kg/m^3 to densities of 16.2, 32.4, 64.8, 97.2, or 194.4 kg/m^3, there were significant elevations in blood glucose levels of fish transferred to the higher densities within 4 hours. Although there was some acclimation within 12 days, the highest density group had elevated glucose levels even after 16 days (Wedemeyer 1976).

Similar transfer of smolting coho salmon resulted in significant increases in blood glucose levels at all new densities, with blood glucose levels of all other density groups significantly greater than that of 16.2 kg/m^3 density group. In addition, similar studies conducted on juvenile rainbow trout resulted in an initial increase in blood glucose levels of all density groups, and a return to normal values within 12 days (Wedemeyer 1976).

4. Season. Seasonal variations have been found in the glucose levels of some teleosts, including killifish. During a 20-month study on field populations of killifish, glucose concentrations were at peak levels during June to July and September to May. The former period appeared to coincide with the peak of the spawning season, and the glucose levels during this period were not correlated with water temperature. However, the glucose levels during the latter period appeared to be inversely correlated with water temperatures (Leach and Taylor 1976).

Seasonal variations were also found for glucose values of other teleosts. Field populations of smallmouth bass, which were studied between June and November, had elevated glucose levels during June to early August and during late August to early September (Shell 1961). Blood glucose levels were higher in winter black bullhead and white crappie acclimated to 5°C than in summer fish of the same species acclimated to 20°C (Dean and Goodnight 1964). Glucose levels of winter bluegill held at 5°C, were significantly lower than summer bluegill held at 20°C (Dean and Goodnight 1964). In a similar experiment, however, there were no significant differences between the glucose levels of winter and summer largemouth bass (Dean and Goodnight 1964).

5. Temperature. Temperature studies have also been performed on glucose levels of some teleosts. Cold exposure of freshwater killifish resulted in elevated glucose levels (Umminger 1971). Abrupt transfer of channel catfish acclimated at 30° to 10°C resulted in elevated glucose levels. When the cold-shocked fish were returned to 30°C, the glucose levels returned to normal within 24 hours (Block 1974).

The effect of elevated temperatures on glucose levels was examined in brook trout. No significant temperature effects were found for the glucose levels of starved or meat-fed brook trout, although glucose levels were higher at elevated temperatures in meat-meal-fed brook trout (Phillips 1961).

6. Strain. Comparison of wild and cultured rainbow trout (Idaho and Shasta) revealed similar values for blood glucose levels (Barnhart 1969). However, McCarthy et al. (1973) suggested that blood glucose levels were significantly higher in wild rainbow trout than in cultured Idaho, Shasta, and Kamloops strains of trout, whereas the glucose levels of Idaho, Shasta, and Kamloops varieties of rainbow trout were similar (McCarthy et al. 1973).

7. Sex. Little information was found on the variation in glucose levels associated with fish sex. In a study on rainbow trout, Barnhart (1969) found no significant differences between the glucose levels of male and female trout.

C. Protein Concentrations. Variations in the protein concentrations of teleosts may result from differences in environmental conditions or from differences inherent to the fish population examined. The effects of some potential sources of variation are discussed below.

1. Capture and transport. Field-captured teleosts may have different serum protein levels than hatchery-reared or laboratory-acclimated fish due to effects of capture and transportation. Wild rainbow trout had significantly higher serum protein levels than hatchery-reared rainbow trout (Barnhart 1969). On the other hand, there were no significant differences in protein levels between field-captured and laboratory-acclimated shiners (Courtois 1975a). Freshwater mudfish exposed to capture and transportation stress had reduced plasma protein levels within the first 2 to 3 days (Hattingh and Van Pletzen 1974). These values returned to normal within 7 to 10 days.

2. Anesthesia. Another potential source of variation, which was examined in rainbow trout, is anesthetization of fish. Electroshock anesthesia did not appear to cause variations in the plasma protein levels of rainbow trout (Schreck et al. 1976).

3. Diet. Diet has also been investigated as a possible source of variation in fish plasma protein levels. Diet did not appear to alter plasma protein levels in rainbow trout fed either Colorado or Santa Monica diets (Barnhart 1969).

4. Season. Seasonal effects on serum protein levels have been investigated in carp, rainbow trout, and smallmouth bass. There were significant differences in protein levels of carp acclimated to low temperatures during the summer, fall, and winter, but not in carp acclimated to high temperatures (Houston et al. 1970). Rainbow trout held at 11-13°C had significantly higher plasma protein levels in the winter and summer than in the spring (Denton and Yousef 1975). The plasma protein levels of goldfish were significantly decreased during June to August. However, this variation may have been related to weight, sexual development, or nutritional changes (Summerfelt et al. 1967).

The total serum protein levels of smallmouth bass appeared to be reduced from June to mid-August and to be elevated from mid-August to mid-November (Shell 1961). Cyclical variations in serum globulin levels appeared to account for much of the variation in total protein levels of smallmouth bass during June to November. The albumin levels were almost stable from June to late September and then appeared to increase until mid-November. The albumin-globulin ratio appeared to be inversely related to globulin and total serum protein levels (Shell 1961).

5. Temperature. The effect of temperature on protein levels has been studied in some teleosts. Exposure of channel catfish to abrupt cold shock resulted in decreased protein levels, which did not return to previous levels within 48 hours (Block 1974). Total protein levels were also significantly lower in fall carp acclimated to 4°C than those acclimated to 17°, 27°, and 33°C (Houston et al. 1970, Houston and Madden 1968). However, there were no significant differences in protein levels of summer carp acclimated to 2° and 7°C nor in winter carp acclimated to 7° and 27°C (Houston et al. 1970).

Only one study was found on the effect of temperature on serum protein components. There were significant differences found between the serum protein component levels of rainbow trout acclimated to 8°C and those of trout acclimated to 16°C (Meisner and Hickman 1962).

6. Age and sex. Other possible sources of variation in plasma protein levels were fish age and sex. The age of the fish appeared to result in significant changes in plasma protein levels in Idaho and Shasta strains of rainbow trout (Barnhart 1969). On the other hand, no significant differences in plasma protein levels were found between male and female rainbow trout (Barnhart 1969).

D. Hemoglobin Concentration. Differences in blood hemoglobin levels may be associated with the population of fish studied and/or with environmental conditions used. Some effects of the potential sources of variation in hemoglobin levels are examined below.

1. Capture, transport, handling, and anesthesia. Comparative studies were done between field-captured and laboratory-acclimated fish. The hemoglobin levels of field-captured golden shiners were not significantly higher than those of laboratory-acclimated fish (Courtois 1975a). However, capture and transportation of field freshwater mudfish resulted in decreased hemoglobin levels within the first 2 or 3 days after capture. These values returned to normal within 7 to 10 days (Hattingh and Van Pletzen 1974). Handling did not significantly alter hemoglobin levels in brook trout, although the use of MS222 anesthesia resulted in elevated hemoglobin levels (Houston et al. 1971).

2. Diet. Another factor which may cause variation in hemoglobin levels is diet. There were significant differences in hemoglobin levels between rainbow trout fed Colorado and Santa Monica diets. In addition, there were significant diet and age interactions (Barnhart 1969).

3. Crowding. Another factor which may cause variations in hemoglobin levels includes loading density. Significantly lower hemoglobin levels were found in goldfish subjected to high loading density (Burton and Murray 1979).

4. Season. Seasonal variations in hemoglobin levels have been found for some teleosts, but not for others. Hatchery-reared lake trout sampled in September had a different frequency distribution of hemoglobin levels less than 5.0 gms/100 ml of blood than lake trout sampled in March (Piper and Stephens 1962). In addition, laboratory acclimation studies indicated that there was a progressive increase in hemoglobin levels of brook trout held at low temperatures from summer to winter, although this seasonal variation was not found at higher acclimation temperatures (Houston and DeWilde 1969). Significantly lower hemoglobin levels were also found in summer rainbow trout than in fall-winter trout at temperatures ranging from 7° to 17°C, whereas no seasonal variations were found in trout held at 3° or 21°C (DeWilde and Houston 1967). However, Denton and Yousef (1975) found that hemoglobin values of rainbow trout held at 11°-13°C were higher during winter and summer than during the spring.

Field studies on hemoglobin values of some fish have also been performed. The hemoglobin levels of mountain whitefish remained relatively constant over a 1-year period, except for April 1961, in which the number of samples obtained was insufficient to determine a seasonal trend (McKnight 1966). On the other hand, the total blood iron levels of smallmouth bass behaved in a somewhat cyclic manner between June to November (Shell 1961).

5. Temperature. The effects of temperature on hemoglobin levels have been examined in some species of teleosts. The results of the studies on the effects of elevated temperatures on hemoglobin values have been conflicting.

When Banks et al. (1971) exposed chinook salmon fingerlings to temperatures ranging from 10°-18°C, only the smallest fingerlings showed any significant temperature effect on hemoglobin levels. These smallest fingerlings had significantly higher hemoglobin levels at 18°C than at 10° or 13°C (Banks et al. 1971).

Hemoglobin levels for many of the studies appear to increase with temperature. Studies on brook trout indicated that temperature increases were associated with increases in hemoglobin levels (Houston and DeWilde

1969). When rainbow trout were acclimated to temperatures ranging from 3° to 21°C during the summer and fall-winter, the hemoglobin levels of the summer trout also appeared to increase at elevated temperatures. In addition, the relationship of hemoglobin levels of fall-winter carp with temperature was in the shape of an ascending curve with a plateau at the intermediate temperatures (DeWilde and Houston 1967). Acclimation of rainbow trout to temperatures ranging from 10° to 18°C also resulted in increased hemoglobin levels as the temperature increased (Houston and Cyr 1974). In addition, similar increases with temperature were found for goldfish acclimated to 20°, and 35°C (Houston and Cyr 1974). When fall carp were acclimated to temperatures ranging from 4° to 33°C, the hemoglobin values also appeared to increase with temperature (Houston and DeWilde 1968). Significant differences in hemoglobin levels were also found between winter carp at 7° and 27°C (Houston and DeWilde 1968).

Exposure of largemouth bass acclimated from 18°-24°C, to temperatures ranging from 5° to 35°C for 2 hours resulted in elevated hemoglobin levels of fish at 30° and 32°-33°C. The mean hemoglobin level of largemouth bass exposed to 30°C was significantly greater than the means of all other groups, including those of bass exposed to 35°C (Denyes and Joseph 1956).

Other studies have found that hemoglobin values decreased with exposure to elevated temperatures. The total blood iron levels of goldfish acclimated at 20°C became markedly variable after transfer to 30°C. The total blood iron levels decreased toward the end of the 10-day holding period after transfer to 30°C (Falkner and Houston 1966). This indicated that a decrease in total blood iron levels may be associated with acclimation to 30°C, which conflicts with the results of Houston and Cyr (1974).

In addition, emaciated bluegill, which were captured in a heated discharge area where water temperatures reach 36°-40°C, had lower plasma iron levels than healthy bluegill, which were captured from cooler areas of the lake. The low plasma iron levels appeared to coincide with low conditon factor of emaciated fish (Graham 1974).

In contrast to the above studies on the effect of elevated temperatures on hemoglobin values, similar hemoglobin values were found in striped bass exposed to 10.8° and 17.5°C (Courtois 1976).

Conflicting results have also been found for the effect of cold exposure on hemoglobin values. No significant differences were found between hemoglobin levels of summer carp held at 2° and 7°C (Houston and DeWilde 1968). Decreased hemoglobin levels, however, were found in goldfish and rainbow trout during cold acclimation (Houston and Cyr 1974).

6. Strain. Differences in hemoglobin concentrations may be found between different populations of fish. Significant differences in hemoglobin levels were found between Idaho and Shasta strains of rainbow trout (Barnhart 1969). There were also interactions between strain and age of the rainbow trout (Barnhart 1969). In addition, wild rainbow trout had significantly higher hemoglobin levels than hatchery-reared Idaho and Shasta strains of rainbow trout (Barnhart 1969). However, no significant differences in hemoglobin levels were found between wild and hatchery-reared lake trout (Piper and Stephens 1962).

7. Age. Fish age or size may also be associated with variations in the hemoglobin levels. There were significant differences in hemoglobin levels of rainbow trout associated with the age of the fish (Barnhart 1969). Das (1964-65) also found that hemoglobin levels of Indian carp appeared to vary with length-at-age. In addition, the only significant temperature effects on hemoglobin levels of fingerling chinook salmon were found in the smallest group of fish (Banks et al. 1971). The hemoglobin levels of brook trout often appeared to be correlated with specimen size also (Houston and DeWilde 1969). In addition, lower hemoglobin levels were found in smaller (weight) mountain whitefish (McKnight 1966). However, goldfish hemoglobin levels appeared to be inversely related to fish length (Burton and Murray 1979). On the other hand, no consistent relationship was found between weight or fish length and hemoglobin levels of rainbow trout (DeWilde and Houston 1967), or between length and hemoglobin levels of smallmouth buffalo fish and bigmouth buffalo fish (Chlebeck and Phillips 1969).

8. Sex. Variations in hemoglobin levels may be associated with fish sex in some teleosts. The hemoglobin levels of male goldfish appeared to be significantly higher than those of female fish (Summerfelt 1967, Burton and Murray 1979). In addition, male mountain whitefish had significantly higher hemoglobin values than female fish (McKnight 1966). However, there were no significant differences between male and female hemoglobin levels in rainbow trout (Barnhart 1969), smallmouth buffalo fish, and bigmouth buffalo fish (Chlebeck and Phillips 1969).

E. Hematocrit Levels. Variations in the hematocrit levels of teleosts may be associated with the population of fish examined and/or with environmental conditions. The effects of some potential sources of variation are discussed below.

1. Capture, transport, handling, and anesthesia. Similar hematocrit values between field-captured and laboratory-acclimated fish were found for golden shiners (Courtois 1975a), and catostomid fish (Powers 1974). On the other hand, freshwater mudfish subjected to capture and transportation stress had reduced hematocrit levels within the first 2 to 3 days, but these returned to normal within 7 to 10 days (Hattingh and Van Pletzen 1974).

There was no significant variation in the hematocrit levels of brook trout following handling, although anesthesia with MS222 resulted in elevated hematocrit levels (Houston et al. 1971). On the other hand, electroshock anesthesia appeared to have no significant effect on hematocrit levels in rainbow trout (Schreck et al. 1976).

2. Diet. Another factor which was studied is the effect of diet on hematocrit levels. Diet appeared to influence the hematocrit levels of rainbow trout fed either Santa Monica or Colorado diets (Barnhart 1969).

3. Crowding. Hematocrit levels of fish may also be influenced by other experimental conditions, including fish loading density and fish diet. Goldfish held at a high loading density had significantly lower hematocrit levels than controls (Burton and Murray 1979).

4. Season. Some authors have found seasonal variations in hematocrit levels. Hematocrit levels of field-captured catostomid fishes were elevated during February to July during the first year and March to August during the next year, and were reduced during September to December for both years. The lowest hematocrit values of catostomid fishes corresponded with the lowest water temperatures and the higher hematocrit values corresponded with higher water temperatures (Powers 1974). In addition, the hematocrit levels of brown bullhead and channel catfish decreased during the summer, when these species approached full breeding conditions (Haws and Goodnight 1962). Seasonal differences in hematocrit levels were also found in summer, winter, and fall carp held at lower acclimation temperatures, but not in fall and winter carp held at higher temperatures (Houston and DeWilde 1968).

Seasonal studies were also performed on salmonid fishes. Field-captured mountain whitefish had elevated hematocrit levels during April and July to September and no other seasonal variations were found (McKnight 1966). In addition, the hematocrit levels of rainbow trout held at 11°-13°C were lower during the spring than during the summer and winter (Denton and Yousef 1975). When rainbow trout were acclimated to temperatures ranging from 3° to 21°C during the summer and fall-winter, seasonal differences in hematocrit levels were also found at 11° and 14°C (DeWilde and Houston 1967).

In addition, there were differences in the frequency distribution of hematocrit values in cultured lake trout sampled in September as compared with those of trout sampled in March. These differences may be related to dietary variations during the year (Piper and Stephens 1962).

5. Temperature. Temperature may also influence the hematocrit levels of teleosts. Many studies have been performed to determine the effect of elevated temperatures on hematocrit values of teleosts. The results, however, have been conflicting.

Many studies on salmonids have found little variation in hematocrit levels associated with elevated temperatures. The hematocrit levels of rainbow trout held at 5°C were not significantly different than those of trout held at 15°C (Weber et al. 1976). Normandeau (1962) also found no significant correlation between water temperature and the mean hematocrit values of lake trout, splake trout, rainbow trout, brook trout, and landlocked salmon. In addition, no significant variations were found in hematocrit levels of presmolt coho salmon exposed to temperatures ranging from 1.3° to 15.0°C (Giles and Vanstone 1976).

Studies on other species of teleosts have also found little variation in hematocrit levels associated with elevated temperatures. No significant differences were found between winter carp acclimated to 7° and 27°C (Houston and DeWilde 1968). In addition, fall carp acclimated to temperatures ranging from 4° to 33°C had no variation in hematocrit levels at the higher temperatures (Houston and DeWilde 1968).

No significant variations were found in goldfish held at 21.5°, 10°, and 5°C (Catlett and Millich 1976). Abrupt transfer of goldfish acclimated at 20° to 30°C also did not appear to significantly alter hematocrit levels during a 10-day period in which fish held at 30°C, except at 60 and 80 hours post shock (Falkner and Houston 1966). In addition, the hematocrit levels of striped bass were similar between 10.8° and 17.5°C (Courtois 1976).

Many studies on salmonids have revealed that elevated hematocrit levels may be associated with increased temperatures. The hematocrit levels of 22°C-acclimated rainbow trout were significantly greater than those of 5°C-acclimated trout (Weber et al. 1976). Except for the 11°C group, the hematocrit levels of summer rainbow trout acclimated to 21°C were significantly greater than those of fish acclimated to temperatures below 21°C (DeWilde and Houston 1967). The hematocrit levels of rainbow trout, acclimated to temperatures of 2°, 10°, and 18°C, also appeared to increase with temperature (Houston and Cyr 1974). In addition, there appeared to be a general increase in hematocrit levels of brook trout with acclimation temperature over the range of 2° to 20°C (Houston and DeWilde 1969). Finally, when chinook salmon fingerlings were acclimated to temperatures ranging from 10° to 18°C, the only significant temperature effects on hematocrit values were found in the smallest fingerlings. In this group, the hematocrit levels appeared to generally increase with temperature (Banks et al. 1969).

Studies on other species of teleosts also revealed that increased hematocrit levels may be related to elevated temperatures. Field studies on catostomid fish revealed that hematocrit values were increased at elevated temperatures and were reduced at lower environmental temperatures (Powers 1974). Laboratory studies confirmed that the hematocrit levels of catostomid fish appeared to increase with temperature (Powers 1974). In addition, when goldfish were acclimated to 2°, 20°, and

35°C, the hematocrit levels appeared to increase at higher temperatures (Houston and Cyr 1974). Finally, lower hematocrit levels were found in goldfish held at 1°C than in fish held at 21.5°C (Catlett and Millich 1976).

Only one study was found in which reduced hematocrit values may be associated with elevated temperatures. The hematocrit levels of emaciated bluegill, captured from the heated discharge area of Lake Julain, were lower than those of healthy bluegill captured from cooler areas of Lake Julian. The low hematocrit levels were highly correlated with lower condition factors of emaciated fish. These results may be caused by high temperatures limiting the abundance of food organisms and thereby inducing anemia (Graham 1974).

Variable results were also found on the effect of cold exposure on hematocrit levels. No significant differences in hematocrit levels were found between summer carp acclimated to 2° and 7°C (Houston and DeWilde 1968). However, fall carp acclimated to temperatures ranging from 4° to 33°C had reduced hematocrit levels at the lower temperatures (Houston and DeWilde 1968). In addition, cold shock of 20°- to 25°C-acclimated goldfish rsulted in significant reductions in hematocrit levels (Platner 1950).

The effect of cold shock was also studied in channel catfish. The abrupt transfer of catfish acclimated at 30° to 10°C resulted in a significant decrease in hematocrit levels within 24-48 hours after transfer. Hematocrit levels returned to normal within 1 hour after the goldfish were returned to 30°C. After the first hour the hematocrit levels decreased and remained reduced throughout the remainder of the 96-hour period (Block 1974). DeWilde and Houston (1967) also found that the hematocrit levels of summer and fall-winter rainbow trout were lower at colder temperatures.

6. Strain. One source of intraspecific variation in hematocrit levels was the fish population sampled. The hematocrit levels of Shasta strain of rainbow trout was significantly lower than those of the Idaho strain of trout (Barnhart 1969). When these strains of trout were compared with the Kamloops strain, the Kamloops strain had significantly higher hematocrit levels (McCarthy et al. 1973). The hematocrit levels of wild rainbow trout were similar to those of the Kamloops strain (McCarthy et al. 1973) but were significantly higher than the hematocrit levels of the Idaho and Shasta strains of cultured trout (Barnhart 1969). When hematocrit values for salmonids in New Hampshire hatcheries were compared for any given period of time, there were significant, but inconsistent, intraspecific differences, which may have been due to other factors (Normandeau 1962). However, when lake trout from different hatcheries were compared during the same time of year, similar hematocrit levels were found (Piper and Stephens 1962).

7. Age or size. Conflicting results were found for the relationships between fish age or size and hematocrit levels of different teleosts. Barnhart (1969) found that age appeared to result in variations in hematocrit levels of cultured rainbow trout. In addition, there were significant strain-age, diet-age, and sex-age interactions. The hematocrit levels of brook trout increased over a 3-month period as the age increased from 6 to 8 months (Normandeau 1962). Fingerlings and 1- to 2-year-old brook trout had significantly lower hematocrit levels than production brook trout. However, this may have been due to a high incidence of disease (Snieszko 1961). No significant differences were found between the fingerling and production trout hematocrit levels of rainbow trout or brown trout (Snieszko 1961).

There was no consistent relationship between fish length and hematocrit levels of teleosts. No consistent significant trends were found between length and hematocrit levels of smallmouth buffalo fish and bigmouth buffalo fish (Chlebeck and Phillips 1969), or between fish length and hematocrit levels of rainbow trout (DeWilde and Houston 1967). The hematocrit levels of goldfish appeared to be inversely correlated with fish length (Burton and Murray 1979).

There appeared to be no consistent relationships between weight and hematocrit levels of rainbow trout (DeWilde and Houston 1967). However, a significant correlation was found between weight and hematocrit levels of brook trout held at 2°C, although this was not a general feature (Houston and DeWilde 1969). Hematocrit levels appeared to vary with the weight of channel catfish and brown bullhead (Haws and Goodnight 1962), and mountain whitefish (McKnight 1966). In addition, temperature effects on hematocrit levels of chinook salmon fingerlings were found only in the smallest fish (Banks et al. 1971).

8. Sex. Hematocrit values may also vary with the sex of the fish. Male goldfish appeared to have significantly higher hematocrit levels than the female fish (Burton and Murray 1979, Summerfelt et al. 1967). Male mountain whitefish also had higher hematocrit levels than females (McKnight 1966). Significant differences in hemtocrit values of male and female fish were also found in sexually mature rainbow, brook, and brown trout (Snieszko 1961). However, no significant differences in hematocrit levels were found between male and female smallmouth buffalo fish, bigmouth buffalo fish (Chlebeck and Phillips 1969), and cultured rainbow trout (Barnhart 1969, Schreck et al. 1976).

F. Hematological Indices. Differences in the hematological indices may be associated with variations in the fish population studied and/or with variations in the environmental conditions used. Potential impacts of some sources of variation are discussed below.

1. Crowding. The effect of loading density on the hematological indices was investigated in goldfish. Variations in fish loading density did not appear to alter the mean erythrocytic volume, mean erythrocytic hemoglobin, or mean erythrocytic hemoglobin concentration levels of goldfish (Burton and Murray 1979).

2. Season. Seasonal variations in the hematological indices have been studied in some freshwater teleosts. The mean erythrocytic volumes of rainbow trout were elevated in July and reduced in March (Denton and Yousef 1975). DeWilde and Houston (1967) found that seasonal differences between summer and fall-winter rainbow trout were found only at 7°C, but not at other temperatures. No significant differences were found in the mean erythrocytic volume of carp at moderate to high temperatures, but significant seasonal differences were found at lower temperatures (Houston and DeWilde 1968).

The mean erythrocytic hemoglobin values of rainbow trout appeared to be higher in January than in March (Denton and Yousef 1975). In addition, Houston and DeWilde (1968) found that fall carp had significantly lower mean erythrocytic hemoglobin levels than summer and winter carp.

Denton and Yousef (1975) found that the mean erythrocytic hemoglobin concentration of rainbow trout was elevated in April and reduced in July. However, DeWilde and Houston (1967) found that there were no significant differences between rainbow trout during the summer and fall-winter trout except at 7° and 17°C. On the other hand, the mean erythrocytic hemoglobin concentration of winter carp appeared to be greater than those of summer or fall carp at intermediate water temperatures (Houston and DeWilde 1968).

3. Temperature. The influence of temperature on the hematological indices has been studied in some teleosts. The results of the effects of elevated temperatures on mean erythrocyte volume are conflicting. Goldfish acclimated to 10°C had significantly lower mean erythrocytic volumes than goldfish acclimated to 21.5°C (Catlett and Millich 1976). In addition, abrupt transfer of goldfish acclimated at 20° to 30°C resulted in an initial decrease in mean erythrocyte volume. These values returned to normal in goldfish held at 30°C for 10 days post-heat shock (Falkner and Houston 1966). However, the mean erythrocytic volume of winter, summer, and fall carp was not influenced by temperature at the elevated temperatures (Houston and DeWilde 1968). In addition, the mean erythrocytic volume of brook trout or fall-winter and summer rainbow trout did not appear to vary markedly with acclimation temperatures over the approximate range of 2° to 21°C (Houston and DeWilde 1969, DeWilde and Houston 1967).

Contradictory results were also found on the effects of cold exposure on mean erythrocytic volume. The mean erythrocytic volume of winter, summer, and fall carp was inversely correlated with temperature at the colder temperatures (Houston and DeWilde 1968). However, mean erythrocytic volumes of 1°- and 5°C-acclimated goldfish were not significantly different than those of 21.5°C-acclimated fish (Catlett and Millich 1976).

Few studies were found on the effect of elevated temperatures on mean erythrocytic hemoglobin levels, and these appeared to have variable results. Significant differences in mean erythrocytic hemoglobin levels were found between fall carp acclimated to 27°C and those acclimated to 4° and 33°C. Significant differences were also found between 7°C-acclimated and 27°C-acclimated winter carp (Houston and DeWilde 1968). However, mean erythrocytic iron content remained relatively stable when 20°C-acclimated goldfish were abruptly transferred to 30°C and kept at 30°C for 10 days (Falkner and Houston 1966). There were also no significant differences in mean erythrocytic hemoglobin levels of fall carp held at 4°, 17°, and 33°C (Houston and DeWilde 1968).

The effects of cold temperature on mean erythrocytic hemoglobin levels were examined in few species of teleosts. When brook trout were acclimated to temperatures of 2°, 5°, 8°, 10°, and 20°C, only the group acclimated to 5°C had mean erythrocytic hemoglobin levels significantly different from those of any other group (Houston and DeWilde 1969). However, the mean erythrocytic hemoglobin levels did not appear to vary significantly between summer carp held at 2° and 7°C (Houston and DeWilde 1968).

Mean erythrocytic hemoglobin concentrations of teleosts may be influenced by temperature. The mean erythrocytic hemoglobin concentration of fall-winter and summer rainbow trout were investigated over a temperature range of 3° to 21°C. The mean erythrocytic hemoglobin concentration of fall-winter rainbow trout acclimated to 3°C was significantly less than those of all other groups. The mean erythrocytic hemoglobin concentration of summer trout acclimated to 21°C was significantly higher than those of fish acclimated to 3°, 7°, 11°, and 17°C (DeWilde and Houston 1967). However, the hemoglobin concentration within erythrocytes of catostomid fish did not appear to change significantly with acclimation temperature (Powers 1974).

4. Size and sex: Neither fish size nor sex was associated with variations in the hematological indices of several species of teleosts. There were no consistently significant trends found between fish length and the hematological indices of goldfish (Burton and Murray 1979). No significant trend was found between fish length or weight and the mean erythrocytic volume or mean erythrocytic hemoglobin concentration of rainbow trout (DeWilde and Houston 1967). In addition, there was no significant variation in the mean erythrocytic hemoglobin of smallmouth buffalo fish or bigmouth buffalo fish associated with fish length or sex (Chlebeck and Phillips 1969).

G. Erythrocyte Sedimentation Rates. Very few studies were found on the erythrocytic sedimentation rates of fish blood. However, some potential sources of variation will be discussed below.

1. Diet and crowding: Some experimental conditions which might alter erythrocyte sedimentation rates were also examined. When cultured rainbow trout were fed with Colorado or Shasta diets, there were no significant variations in erythrocyte sedimentation rates associated with diet alone, although significant diet-strain interactions were found (Barnhart 1969). On the other hand, exposure of goldfish to high fish loading density resulted in changes in erythrocyte sedimentation rates (Burton and Murray 1979).

2. Temperatures: Very little information was available on temperature effects on the erythrocyte sedimentation rates of fish blood. A comparative study was performed between emaciated bluegill, captured from the discharge area of a power plant where temperatures reached 36^o-40^oC, and healthy bluegill, captured from cooler water of the same lake. The emaciated fish from the discharge area had lower erythrocyte sedimentation rates than healthy bluegills from the cooler area of the lake. The erythrocyte sedimentation rates were positively correlated with the condition factor, which was lower in emaciated fish from the discharge area (Graham 1974).

3. Strain. One source of intraspecific variation in erythrocyte sedimentation rates is the population of fish studied. Significant differences in erythrocyte sedimentation rates were found between Shasta and Idaho strains of rainbow trout (Barnhart 1969). The erythrocyte sedimentation rates of these strains of rainbow trout were significantly higher than those of the Kamloops variety or wild trout (Barnhart 1969, McCarthy et al. 1973).

4. Age, size, and sex. Variations in age, size, or sex of the fish may also cause variations in erythrocyte sedimentation rates. Age alone had a significant effect on the erythrocyte sedimentation rates of cultured rainbow trout. There were also significant strain-age interactions found (Barnhart 1969). Elevated erythrocyte sedimentation rates were found in mountain whitefish with the lowest weight (McKnight 1966). The erythrocyte sedimentation rate of goldfish appeared to be highly correlated with fish length (Burton and Murray 1979). Significantly lower erythrocyte sedimentation rates were found in male goldfish than in females (Burton and Murray 1979), whereas no significant variations in erythrocyte sedimentation rates were found between male and female mountain whitefish (McKnight 1966) or rainbow trout (Barnhart 1969).

H. Erythrocyte Abundance. Environmental conditions and/or conditions inherent to the population of fish used may cause variations in erythrocyte abundance. Some potential sources of variation are examined below.

1. Capture and transport. One factor which may be associated with variations in erythrocyte abundance is capture and transportation of fish. A decrease in erythrocyte abundance in freshwater mudfish occurred within 2-3 days after capture (Hattingh and Van Pletzen 1974).

2. Crowding and diet. Crowding and diet are other environmental conditions which may be associated with variations in erythrocyte counts. Exposure of goldfish to high loading density resulted in decreased erythrocyte counts of goldfish (Murray and Burton 1979). On the other hand, diet alone had no effect on the erythrocyte levels of cultured rainbow trout fed either Colorado or Santa Monica diets, although significant diet-strain interactions were found (Barnhart 1969).

3. Season. Studies have been found on the effect of season on erythrocyte abundance of some salmonids. Higher erythrocyte counts were found in mountain whitefish during October to December than during other times of the year. The increased erythrocyte abundance appeared to be associated with the spawning period (McKnight 1966). In addition, lake trout sampled in September had a different frequency distribution in erythrocyte abundance than trout examined in March (Piper and Stephens 1962). Significant differences in erythrocyte abundance were also found between summer and fall-winter rainbow trout acclimated to 7° and 17°C, although no significant variations were found between summer and fall-winter trout acclimated to 3°, 11°, 14°, and 21°C (DeWilde and Houston 1967). Denton and Yousef (1975) also found no significant variations in the erythrocyte abundance of rainbow trout held at 11°-13°C during January to November.

Season may also influence erythrocyte abundance of other freshwater teleosts. Decreases in erythrocyte counts of brown bullhead and channel catfish occurred as the summer passed, and these changes also accompanied changes in the breeding condition of the fish (Haws and Goodnight 1962). Significant seasonal variations in the erythrocyte counts of summer, fall, and winter carp were also found at low temperatures, but not at high temperatures (Houston and DeWilde 1968).

4. Temperature. Temperature studies have also been performed on the erythrocyte abundance of salmonids. When chinook salmon fingerlings were exposed to temperatures ranging from 10° to 18°C, the only variations in corpuscle counts associated with temperature were found in the smallest fingerlings. In this group, salmon held at 10° and 13°C had significantly lower corpuscle counts than those held at 16° and 18°C (Banks et al. 1971).

Many of these studies revealed a tendency to have elevated erythrocyte counts at elevated temperatures. The abrupt exposure of juvenile coho salmon to crowding and elevated temperature conditions resulted in an increase in erythrocyte abundance at 12 and 24 hours post shock (McLeay 1975). An increase in erythrocyte abundance with increasing acclimation temperature was also found in brook trout (Houston and DeWilde 1969) and in fall-winter and summer rainbow trout (DeWilde and Houston 1967). The relationship between erythrocyte count and acclimation temperature in rainbow trout appeared to be that of an ascending curve with an intermediate plateau (DeWilde and Houston 1967).

Temperature effects on erythrocyte abundance have also been found in other freshwater teleosts exposed to elevated temperatures. When largemouth bass acclimated from 21° to 25°C were exposed to temperatures ranging from 5°-35°C for periods up to 2 hours, there was a significant increase in the red blood cell count of fish held at 30°C (Denyes and Joseph 1956). In addition, when carp were subjected to temperature acclimation, there appeared to be a general trend toward elevated erythrocyte abundance at elevated acclimation temperatures. Fall carp acclimated to 4° and 17°C had significantly lower erythrocyte counts than those of fish acclimated to 27° and 33°C (Houston and DeWilde 1968).

Other studies revealed little variation in erythrocyte counts associated with elevated temperatures. When goldfish acclimated at 20°C were abruptly transferred to 30°C and held for 10 days, there appeared to be no consistent trend between erythrocyte abundance and heat shock, although significant differences in erythrocyte levels were observed at 60 hours and 144 hours post-heat stress (Falkner and Houston 1966). Also, no significant temperature effects were found in the erythrocyte counts of winter carp held at 7° and 27°C (Houston and DeWilde 1968). In addition, no significant variations were found between fish acclimated to 5°, 10°, and 20°C (Catlett and Millich 1976).

The effect of cold exposure on erythrocyte counts was examined in relatively few studies. The results from these studies were not consistent. No significant differences in erythrocyte abundance were found between summer carp held at 2° and 7°C (Houston and DeWilde 1968). However, acclimation of goldfish to 1°C resulted in decreased erythrocyte counts (Catlett and Millich 1976).

5. Strain. Intraspecific variations in erythrocyte counts may be found between different populations of fish. Significant differences in total blood corpuscle counts were found between Idaho and Shasta strains of rainbow trout (Barnhart 1969). Wild rainbow trout had significantly higher total blood corpuscle counts than Idaho, Shasta, and Kamloops varieties of cultured rainbow trout (Barnhart 1969, McCarthy et al. 1973). However, there were no significant differences in the erythrocyte counts of wild and cultured lake trout (Piper and Stephens 1962).

6. Age and size. Fish age and size may also be associated with variations in erythrocyte abundance. Barnhart (1969) found that variations in the total blood corpuscle counts of cultured rainbow

trout were associated with age alone, as well as with age-strain interactions. In addition, the erythrocyte abundance of Indian carp appeared to increase with increases in length-at-age (Das 1964-65). However, only minor differences in erythrocyte counts were found in mountain whitefish of different weight and age (McKnight 1966).

Increases in weight resulted in elevated erythrocyte counts of channel catfish and brown bullheads (Haws and Goodnight 1962). The erythrocyte levels of brook trout and carp appeared to be correlated with fish size (Houston and DeWilde 1968, 1969). Although there was no consistent significant relationship found between weight or fish length and erythrocyte counts in rainbow trout, the erythrocyte count was usually inversely related to weight and fork-length (DeWilde and Houston 1967). In addition, only the smallest chinook salmon fingerlings showed signs of temperature effects on corpuscle counts (Banks et al. 1971). On the other hand, no obvious trends were found between fish length and erythrocyte abundance in smallmouth buffalo fish or bigmouth buffalo fish (Chlebeck and Phillips 1969).

7. Sex. The effect of sex on erythrocyte counts has been studied in some teleosts. Fish sex did not appear to be associated with variations in erythrocyte abundance of rainbow trout (Barnhart 1969), mountain whitefish (McKnight 1966), bigmouth buffalo fish, or smallmouth buffalo fish (Chlebeck and Phillips 1969).

I. Erythrocyte Morphology. Very few studies were found on potential sources of variation in erythrocyte morphology. Some of these sources are discussed below.

1. Crowding. Environmental conditions, such as crowding, may be associated with differences in erythrocyte morphology. There were significant decreases in erythrocyte length, width, and area, with no significant change in erythrocytic shape in goldfish exposed to a high fish loading density (Murray and Burton 1979).

2. Age, size, and sex. Intraspecific differences in erythrocyte morphology may also be associated with fish age, size, or sex. Intraspecific differences in erythrocyte morphology were found to be associated with the weight and age of mountain whitefish (McKnight 1966). Similar erythrocyte sizes were found between male and female mountain whitefish (McKnight 1966).

J. Leucocyte-Thrombocyte Abundance. Variations in the leucocyte-thrombocyte counts of teleosts may be associated with differences in the environmental conditions or with differences in the population of fish under examination. Some of these sources of variation are discussed below.

1. Capture and transport. One source of variation in leucocyte and thrombocyte counts is capture and transport. Capture and transport

of mudfish resulted in reduced leucocyte counts and elevated thrombocyte counts within 2 to 3 days after capture. These values returned to normal within 7 to 10 days (Hattingh and Van Pletzen 1974).

2. Crowding. Another source of variation in leucocyte-thrombocyte counts is crowding. The leucocyte-thrombocyte counts of goldfish decreased significantly upon exposure to crowding (Murray and Burton 1979).

3. Season. Few studies were found on the effect of season on leucocyte-thrombocyte counts. The leucocyte-thrombocyte counts of mountain whitefish were significantly decreased during January to March (McKnight 1966).

4. Age or size. The effect of age or size on leucocyte-thrombocyte counts was investigated in few species of teleosts. Leucocyte-thrombocyte counts did not appear to vary with size in bigmouth buffalo and smallmouth buffalo (Chlebeck and Phillips 1969). Although no size comparisons were made on leucocyte counts of mountain whitefish, there were slight reductions in the thrombocyte counts of younger and smaller fish (McKnight 1966).

5. Sex. Differences in leucocyte-thrombocyte counts associated with fish sex were investigated in a limited number of freshwater teleosts. No significant variations in leucocyte-thrombocyte counts were found between male and female bigmouth buffalo, smallmouth buffalo (Chlebeck and Phillips 1969), and mountain whitefish (McKnight 1966).

K. Cellular Distribution. Experimental variations or variations inherent to the population of fish studied may result in differences in cellular distribution. The effects of some of these sources of variation are discussed below.

1. Anesthesia. Intraspecific variations in leucocyte and thrombocyte abundance may be associated with anesthetization of fish. Exposure of rainbow trout to electroshock anesthesia resulted in an increase in the thrombocyte to total leucocyte-thrombocyte ration and the percentage of heterophils (granulocytes) (Schreck et al. 1976).

2. Season. Little information was found on the effect of season on blood cellular distribution in teleosts. The proportion of each cell type to the total number of leucocyte-thrombocyte cells may vary with the time of year. Leucocyte differentials revealed that the percentage of neutrophils in mountain whitefish was elevated during July to September and was decreased from January to March (McKnight 1966). No other studies were found on seasonal variations in cellular distribution.

3. Age and sex. Intraspecific variations in cellular distribution may also be associated with fish age or sex. One-year-old mountain whitefish had a lower percentage of granulocytes than older fish (McKnight

1966). In addition, leucocyte differentials revealed that male mountain whitefish had a lower percentage of granulcytes than female mountain whitefish (McKnight 1966).

L. Summary and Conclusions. From the above information, it is clear that more studies need to be done to determine potential environmental effects on blood parameters. It is also important to further understand the intraspecific variations in blood parameters due to factors inherent to the fish population examined (strain, age, size, sex, etc.). The information on these sources of variation have been relatively limited for any one blood parameter and for any species of freshwater teleosts. Considerably more information is needed on the effects of these potential sources of variation in blood parameters.

The available information reported here, however, can be of some value. Some of the sources of variation in fish blood parameters were discussed above. When baseline or experimental studies are initiated, caution should be used to control the known sources of variation for the blood parameters being studied.

SECTION 2

CONCLUSIONS

A review of the literature shows that there is a general lack of information on any one blood parameter for freshwater teleosts. More studies are needed to determine normal (nonstressed) blood parameter values for freshwater teleosts. In addition, very little is known of the hematology for any one species of fish. For this reason no baseline levels can be firmly established for any one species of teleosts. The available information also indicates that there may be interspecific and intraspecific variations in the reported values. More information is also needed on the potential sources of these variations so that these factors may be taken into account when trying to establish baseline (nonstressed) blood values.

Standard clinical methods, with some modifications for small blood samples, are adequate for fish hematological studies.

A density stress syndrome seems to exist for goldfish, catfish, and bluegill. Blood dyscrasias associated with density stress include smaller and rounder blood cells, hyperglycemia, an increase in albumin/protein ratios, and decreased values for hematocrit and hemoglobin.

Variations in hematology were observed in adult bluegill depending on the culture complexity. However, results indicated that monoculture data were valid to use as controls as well as for use in bioassays to characterize potential environmental perturbations.

Bluegill hematology was examined in order to determine baseline values. The study of fish blood is complicated by sex and/or seasonal differences. In addition, the selection of hematological indicators is somewhat difficult since 10 blood parameters accounted for approximately 80 percent of the total variation. In general, good hematological indicators appear to be those associated primarily with red blood cell morphology and secondarily with thrombocyte-leucocyte distribution.

SECTION 3

RECOMMENDATIONS

If hematological data are to be used to assess water quality conditions, it is important to minimize extraneous environmental effects. From the information gathered in this study it appears that data from fish held in monocultures would adequately characterize the hematology. It is recommended that fish be stocked at approximately 0.80 fish centimeters per liter of water to obviate possible density stress responses. Since the baseline (or nonstressed) values appear to be complicated by sex and/or seasonal influences, bioassays should include both sexes over the various seasons.

Standard clinical methods, with some modifications to accomodate small blood volume samples, are recommended for hematological studies of teleosts.

SECTION 4

EXPERIMENTAL PROCEDURES

I. Fish Sources

Most fish for these studies were provided by the Carbon Hill National Fish Hatchery located at Carbon Hill, Alabama, where fish were bred, seined from the ponds, and placed in concrete holding tanks for at least four weeks prior to sampling. Water flow was adjusted so that a complete exchange of the 1,514 liter tank volume was achieved in at least 24 hours. Tanks were aerated continuously. The fish were exposed to natural photoperiods and seasonal temperatures. Fish were fed *ad libitum* daily a diet of Purina Trout Chow.

II. Blood Sample Collection

At the end of the acclimation period, fish were caught by dip net, rapidly measured, and weighed. To facilitate blood extraction, fish were placed in a special holder and pithed (Murray and Burton 1977). Blood was collected from the exposed caudal vein according to the blood extraction method detailed by McKnight (1966). A heparinized syringe and microhematocrit tubes were used to extract the blood.

III. Hematological Techniques

Since blood volume is small in fish, approximately 2 percent of the total body weight (Yoffey 1929), micro-clinical methods were necessary.

A. *Hematocrit*. Hematocrit (Hc) is the percentage of packed red blood cells to total blood volume (Hesser 1960). Blood is collected in a microhematocrit capillary tube and centrifuged for five minutes in a hematocrit centrifuge. The method is quite reliable and gives information on the variations in cell volume regardless of blood cell sizes or numbers (Young 1949). The hematocrit may be a useful indicator of anemia, as expressed by the mean erythrocyte volume (MEV), and mean erythrocyte hemoglobin concentration (MEHc). A further use of the hematocrit is that it may indicate fluid shifts into or out of the plasma (Stanley and Colby 1971).

B. *Erythrocyte Sedimentation Rate*. Erythrocyte sedimentation rate (ESR) is the rate in which blood cells settle in a vertical column of blood after standing one hour. The micro-Wintrobe method, described by Blaxhall and Daisley (1973), was used to determine the percent ESR. This is a nonspecific reaction indicating the intensity of a disease process and may be useful in identifying the intensity of stress. ESR was determined by the following relationship:

$$\text{ESR} = \frac{\text{total blood volume} - \text{sedimented blood volume}}{\text{total blood volume}} \times 100$$

C. Hemoglobin. Measurement of hemoglobin levels have been routinely used in testing for the presence of anemia in fish. Several methods have been used to determine hemoglobin levels. Although many of these methods require small amounts of blood, the majority are time-consuming and also may require measured dilutions, volumetric measurements of blood, or standard solutions for colorimetric comparisons. We have found that the Spencer modification of Kennedy's method (Jones 1962) works well for fish blood. It is a rapid and reproducible colorimetric determination using a hemoglobinometer.

D. Blood Cell Enumeration. Blood cell enumeration was determined by microscopy. The hemocytometer counting chamber method was used for enumeration of red blood cells (Jones 1967, Frankel et al. 1970). The blood was drawn into a red blood cell diluting pipette, diluted, and stained with Shaw's solution (Hesser 1960). Red blood cell (rbc) enumeration is important in providing an index of anemia when used with Hc and/or Hb, as follows:

$$\text{MEV} = \frac{\text{Hc} \times 10}{\text{rbc}}$$

$$\text{MEH} = \frac{\text{Hb} \times 10}{\text{rbc}}$$

$$\text{MEHc} = \frac{\text{Hb} \times 100}{\text{Hc}}$$

E. Osmolality. Osmolality of the plasma may be an indication of the ability of teleosts to regulate osmotic concentrations within the body. Osmotic concentration represents the effective concentration of all solutes in the plasma. Plasma osmolality (PO), expressed in milliosmoles per kg, was measured with 5 ml of plasma in a Wescor dewpoint microosmometer.

F. Glucose Concentration. Glucose values in the blood are maintained at a constant level achieved by a balance between glycolysis and glycogenesis. In fish blood, glucose measurements have been used to index physiological stress (Houston et al. 1971, Wedemeyer 1976). The glucose concentrations of the plasma samples were determined with an automatic analyzer (Barnhart 1969). A modification of the glucose oxidase-peroxidase method (Scalvo Glucose Test, Scalvo Incorporated) was used to determine the glucose concentrations of the samples. The glucose concentration was measured in grams per 100 milliliters of blood.

G. Total Protein Concentration. The total protein values represent the sum of the albumin and globulin components. These components are essential in transporting red blood cells, in maintaining body pH and osmotic balance, in regulating cellular activity and function, in contributing to the defense mechanisms against disease and injury, in contributing to nitrogen needs, and in contributing to the protein reserve (Booke 1964). The total protein concentrations of plasma samples were determined using an AO Total Solids-Meter (American Optical), which is essentially a Goldberg refractometer (Houston et al. 1971, Denton and Yousef 1975, Courtois 1975, 1976). The refractometer was previously calibrated using 25 percent normal human albumin (Albuminar 25, Armour Pharmaceutical Company). The total protein level was read in grams per 100 milliliters of blood.

H. Albumin. Albumin usually comprises the major serum component of the total proteins and differs from the globulins in function, metabolism, and site of origin. Osmotic regulation is one of the main functions of albumin. Albumin regulates blood volume in aquatic vertebrates (Poluhowich and Parks 1972). The plasma samples were analyzed for albumin concentration with an automatic analyzer. A bromocresol green method (Centrifichem Test Procedure for Albumin, Union Carbide Corporation Diagnostics) was used to determine the albumin concentrations of the plasma samples (Dow and Pinto 1969, Doumas et al. 1971). Twenty-five percent normal human serum albumin samples (Albuminar 25, Armour Pharmaceutical Company) were used as standards. The albumin concentration was expressed as milligrams per 100 milliliters of blood.

I. Percentage of Albumin to Total Protein. The percentage of albumin to total protein indicates the level of total protein in the blood that is attributable to the albumin. When calculating this percentage, the protein concentration was first converted to milligrams per 100 milliliters of blood. Then the following formula was used:

$$x = \frac{\text{Albumin concentration}}{\text{Total protein concentration}} \times 100$$

The albumin to total protein ratio was expressed as percentage.

J. Differential Counts. Differential counts were determined from dry blood smears stained with Wright's stain. The relative percentage of each cell type present gives some indication of physiological condition. These percentages were determined from a field of 200 cells. This figure was determined from tables based on volumetric cell counts (Arkin and Colton 1963). Classification of the cell types followed the nomenclature of Watson et al. (1963).

K. Cell Morphology. The biological significance of red blood cell morphology is at least two-fold: (1) cell size determines the available volume for oxygen transport, gaseous exchange, and transport of nutrients and biochemical products, whereas (2) cell shape, which influences the surface to volume ratios, determines, in part, the rates of cellular exchanges. Blood smears of either heparinized or non-heparinized blood were stained with Wright's stain. Two hundred blood cells were measured for each fish. This number was determined from sample size statistical tables, based on the volumetric wet cell counts (Arkin and Colton 1963). Cell length and width were measured either with a filar micrometer or from enlarged photographs. Erythrocyte shape, or roundness, was calculated from width/length ratios. Erythrocyte area was based on the area of an ellipse, which closely approximates the shape of red blood cells in fish. Cell height was determined from the equation of an ellipsoid volume.

IV. Goldfish Density Study

Adult goldfish, *Carassius auratus* L., were raised, held, and analyzed at Carbon Hill National Fish Hatchery in Carbon Hill, Alabama. Two holding densities were used in this study. Group 1 consisted of 60 fish held in 1,514 liters of water in a concrete tank, thus giving a holding density of 0.80 fish cm per liter of water. This is considered a desirable density (Lewis 1963). Group 2 consisted of 240 fish held in a 1,514 liter tank, giving a holding density of 4.19 fish cm per liter of water. The fish in group 1 were held from June to August 1976 before sampling, and those in group 2 were held from December 1975 to September 1976. The holding tanks were aerated continuously, fresh well water at 17°C was continuously circulated once through the tanks at a rate of 15.14 liters per minute.

Approximately 60 healthy fish, free of epizootics, from each density group were analyzed. Fish from group 1 were analyzed between August 19 and September 16, 1976, and fish from group 2 were analyzed between June 22 and August 5, 1976. Three to ten fish were analyzed during each of the ten analysis sessions for each group.

V. Catfish Density Study

Channel catfish (*Ictalurus punctatus* Rafinesque) were cultured at four different loading densities, ranging from 0.18 kg/m^3 for the controls to 224 kg/m^3, 337 kg/m^3, and 433 kg/m^3 for the intensive cultures. Fish ranged in age from one to four years. Dissolved oxygen was at saturated levels, and water temperatures were similar for all cultures.

Control fish were collected by angling from a commercial fishout pond in northwestern Alabama. The intensive cultures were kept in concrete raceways (15.2 x 1.2 x 1.1 meters) at Gallatin, Tennessee. Water replacement time of the 20,000 liter raceways was about 25 minutes. Raceway fish were caught with a dip-net.

Fish used in this study were free of epizootics and ranged in size from 0.22 kg to 0.45 kg. Samples were collected from February to August 1973. Blood was collected by cardiac puncture. Ten fish from each of the four cultures, with 50 cell measurements per fish, were used to test for density differences.

A nested one-way analysis of variance test was used for the determination. The 95 percent least significant difference test was used to locate differences between the means (Snedecor and Cochran 1968). In addition, measurements from 20 control fish were used to determine differences in sample size. A t-test was used to compare the two groups: 50 measurements vs. 250 measurements.

VI. Culture Complexity Study

Bluegill, *Lepomis macrochirus* Rafinesque, were obtained from Carbon Hill National Fish Hatchery, Carbon Hill, Alabama. Fish for the monospecific fish culture were seined from hatchery ponds and held in concrete tanks in the hatchery tankhouse during September to November 1976. Water volume turnover time for the 1,514 liter tank was approximately 12 hours. Fish were fed ad libitum a daily diet of Purina Trout Chow. Fish were maintained at seasonal temperatures and photoperiods, although the light was somewhat subdued in the tankhouse. Adult fish were bled in November. The more complex cultures (bi- and tricultures) were located in concrete channels with natural substrates at the Tennessee Valley Authority's Browns Ferry Biothermal Research Station near Athens, Alabama. Water from Wheeler Reservoir was pumped into the outdoor channels. Water volume turnover time for the 530,000 liter channels was approximately 13 hours. The channels simulated self-sustaining aquatic systems. Adult bluegill were stocked in April 1976; walleye and golden shiners were stocked during May and June before the bluegill started to spawn. Fish were cultured under natural temperatures and photoperiods. Fish were sorted and held in outdoor holding tanks 24 hours before they were bled, in late October. Composition of the fish cultures is summarized in Table 16. The densities of adult bluegill and walleye were those initially stocked; however, other densities were continually changing during the course of the study. The numbers of young-of-year bluegill and golden shiners are estimates based on biomass at the termination of the study, when the bluegill were sampled.

Blood from 15 fish from each of the five treatments was examined by multi- and univariate analysis of variance procedures. To test for validity of using monoculture data as controls or in bioassays, data from the monoculture group (group 1) were compared with data from the group comprised of 150 adult bluegill and 25 walleye (group 2), a population ratio which may be considered natural. In order to determine the effects of culturing on bluegill blood all five groups were analyzed. Effects of stocking density of the second species were determined by examining groups 2, 3, and 4, comprised of 150 bluegill and 25, 50, and 100 walleye, respectively. The effects of a more complex culture were determined by comparing data from the monoculture with those from the group comprised of 150 bluegill, 50 walleye, and 10 kg golden shiners (groups 1 and 5), although it was recognized that a possible density effect may exist.

VII. Bluegill Hematology

Adult bluegill (*Lepomis macrochirus* Raf.) were seined from ponds at Carbon Hill National Fish Hatchery, Carbon Hill, Alabama, and kept in concrete holding tanks for approximately one month. Fish were stocked at a biomass density of 3.8 kg/m^3, resulting in 1.1 fish cm/l. Water replacement time of the 1,514 liter tanks was approximately five hours. Temperatures were monitored daily. Although the light was somewhat subdued in the tankhouse, the fish were exposed to a natural photoperiod. Fish were fed ad libitum daily a diet of Purina Trout Chow. Fish were bled about 23 hours after feeding.

Peripheral blood samples were collected in August and November 1977 and February and June 1978. Temperatures ranged from 7.4 ± 2.0°C for February, 12.4 ± 2.2°C for November, 26.0 ± 1.0°C for June, and 29.5 ± 2.2°C for August. Blood collection and most analytical methods are described elsewhere (Burton and Murray 1979, Murray and Burton 1979). In addition, leucocyte-thrombocyte differential counts were determined from a field of 200 cells from blood smear slides, and total plasma protein concentration was measured with an A/O TS-meter.

Twenty males and twenty females were examined during August, November, February, and June. A balanced two-way multivariate analysis of variance was used to test for overall effect of season and/or sex on bluegill hematology. The plasma protein data were not included in these analyses because the data set was incomplete.

SECTION 5

RESULTS AND DISCUSSION

I. Goldfish Studies

A. Blood Chemistry. Comparative results between the two different density groups (Table 5) show significant differences in Hc, Hb, ESR, glucose, and albumin/total protein values. Higher values for Hc (5 percent) and Hb (8 percent) were associated with lower density (group 1), whereas higher values for ESR (71 percent), glucose (23 percent), and albumin/total protein (45 percent) were associated with higher density (group 2). MEV, MEH, MEHc, PO, and total protein and albumin values were virtually the same for both groups.

Many of the hematological values given in Table 5 compare favorably with those reported by others. For example, PO values of 263 mosm/l fall within the 95-percent confidence intervals of values reported by Catlett and Millich (1976). Hc values (38 and 40 percent) are almost the same as other values reported for goldfish (Catlett and Millich 1976, Falkner and Houston 1966, Houston and Cyr 1974). Hb values in our study (9.70 and 10.60 g/100 ml) agree with values for goldfish reported by Falkner and Houston (1966), but are higher than those reported by Houston and Cyr (1974). The MEV value for group 1 (Table 5), 241 cµ, falls within the range of values in goldfish given by Falkner and Houston (1966), although slightly lower MEV values have been reported (Catlett and Millich 1976). In addition, MEH, MEHc, and ESR values fall within the ranges of values reported for other teleosts (McCarthy et al. 1973, DeWilde and Houston 1967, Houston and DeWilde 1968, 1969). The glucose levels of control goldfish in this study were similar to those reported for other freshwater species of teleosts (Field et al. 1943, Houston et al. 1971, Schreck et al. 1976, Umminger 1971, Wedemeyer 1976). Similarly the total protein concentrations of goldfish in this study compared favorably for those of other teleosts (Denton and Yousef 1975, Field et al. 1943, Schreck et al. 1976, Umminger 1969), but were higher than those reported for other goldfish (Summerfelt et al. 1967). On the other hand, the mean albumin concentrations and the mean percentages of albumin to total protein of control goldfish in this study were lower than those reported for other freshwater teleosts (Field et al. 1943, Meisner and Hickman 1962).

Because (1) the fish in group 2 were, on the average, significantly larger (by 26 percent) than those in group 1 and (2) size- or age-related variations in some blood parameters, e.g., Hc, Hb, and rbc abundance, are known to occur in some teleosts (Conroy 1972, DeWilde and Houston 1967, Haws and Goodnight 1962, Houston and DeWilde 1969, Kaplan 1971, McCarthy et al. 1973), the data were further analyzed to identify effects influenced by length. Size-related variations were observed in goldfish for Hc, Hb, and ESR, with an inverse correlation for Hb and Hc (Table 6). Hematological indices, glucose and PO were not influenced by fish size.

Further analysis to factor out influences of length show that length had no influence, except on ESR, when fish were grouped according to sex. Significant sex-related differences existed for Hc and Hb (Table 7). The significant differences in ESR could not be separated from influences of body length in this table. When the data were further stratified by density and sex within each density group, influences of both length and sex became extinct for these responses, except for the inverse L-Hb relationship in group 1, particularly for the females.

Hematological differences related to sex are shown in Table 7. Table 7 indicates that Hc values (40 to 67 percent) and Hb values (10.47 g/100 ml) in males were significantly higher (by about 10 percent) than those found in females. When the data were stratified by density between the sexes, these differences persisted for fish in group 1, but became insignificant for fish in group 2. Although length may have influenced Hb values for the fish in group 1, length probably did not influence Hc values (Table 6). Similarly, ESR values were apparently not related to size within either group. Differences in ESR values in Table 7 were apparently sex-related, since the ESR values were significantly higher (36 to 55 percent) for females than for males in both density groups.

Sex-related differences in Hc and Hb values have been observed for goldfish and other teleosts (Summerfelt 1967, Kaplan 1971, Conroy 1972, McKnight 1966). In these studies, Hc and Hb values were generally higher for males than for females. Summerfelt (1967) reported differences of 10 percent in Hc and Hb values between male and female goldfish. Our results support his findings. Although McKnight (1966) reported no differences in ESR values between males and females of other teleosts, we observed sex-related differences in ESR values for goldfish. The ESR was about 55 percent faster for females than for males. No other sex-related differences were observed for hematological indices and PO.

To identify density-related hematological responses, sexual differences were isolated by grouping the data according to sex (when enough data were available) and comparing the hematological values at each density within each sex. Results of these comparisons are shown in Table 8. When the data were grouped in this way, neither Hc nor Hb values were seen to be influenced by fish length (Table 6). The densities in this study apparently have no effect on Hc and Hb values in the females. Because the apparent decrease (about 7 percent) in Hc for males in group 2 was significant only at the 90-percent probability level, a possible trend is indicated, but not resolved, for effects of density on Hc in males. The test was sensitive enough to resolve a trend for effects of density of Hb in males, since the 10 percent decrease in Hb for males in group 2 was highly significant. Although significant increases in ESR values are indicated for the fish in group 2, these differences are linked to size-related influences (Table 6). To clarify the effect of density on ESR values, data were analyzed from 12 male fish of similar size from each group. The lengths were 20.49 ± 0.32 cm for group 1 and 22.41 ± 0.47 cm for group 2. There were no significant correlations between length and ESR for these 24 fish. ESR values obtained from group 1 were 1.92 ± 9.28 percent and 3.37 ± 1.25 percent for group 2. Because no significant difference exists between these values, there is apparently no effect of density on ESR values in males. There were not enough females of similar size to test for effects of density.

Results from the present study indicate possible effects of density on Hc, Hb, ESR, glucose and albumin/total protein values for goldfish (Table 5). The density effects for Hc, Hb and ESR were apparently masked by fish size and sex (Tables 6 and 7). Despite these factors, the Hc and Hb values were consistently lower for group 2, whereas the ESR values were consistently higher. Although these values were consistent, the Hc and Hb levels did not differ significantly in females. Differences in Hc for males were significant only at the 90-percent level of probability, whereas differences in Hb for males were highly significant. The apparent differences in ESR related to density found for males (Table 8) were rendered insignificant in fish of similar size. Effects of density on ESR values in females could not be determined from this study.

A relatively meager data base exists for the effects of crowding stress on blood chemistry. The results of our study were compared with those of another study (Wedemeyer 1976) investigating density effects on glucose levels of teleosts. In our study, hyperglycemia was found in the blood of goldfish exposed to high population density. Hyperglycemia, resulting from high density conditions, has also been observed for parr and smolting coho salmon and parr rainbow trout.

The consistency of these responses is a significant feature associated with differences in loading densities. Although a consistent trend is obtained to some degree for effects of density on hematology, a more sensitive test apparently is needed to resolve possible differences in some of these blood characteristics.

B. Cell Morphology and Distribution. Volumetric determinations of cell types are summarized in Table 9. All cell types, except hemoblasts and macrophages, were present. Red blood cells accounted for 97 to 98 percent of the total cell count, and thrombocytes accounted for 65 to 83 percent of the remaining cell types. Lymphocytes accounted for 93 to 95 percent of the leucocytes, and other leucocytes, which were not differentiated, accounted for the remaining 5 to 7 percent of the total leucocyte number.

Results in Table 9 indicate different cellular distributions associated with different loading densities. Significantly fewer (9 percent) red blood cells were found in fish held at high density (group 2--1.6 x 10^6 cells/mm^3) than in fish held at low density (group 1--1.8 x 10^6 cells/mm^3). A 35-percent reduction in the abundance of thrombocytes was obtained for fish in group 2, but there were no noticeable differences in clotting time (not measured) between the two groups. About 15 percent more leucocytes were found in fish held at high density, with 14,700 cells/mm^3 in group 2 and 10,100 cells/mm^3 in group 1. Fish in group 2 showed an increase in lymphocytes of about 14 percent and an increase in other leucocytic cells of about 19 percent.

The values obtained for volumetric cellular distributions in our study compare favorably with values obtained by other researchers. Our counts for red blood cells fall within normal ranges reported by other authors (Watson et al. 1963, Falkner and Houston 1966, Spoor 1951, Smith et al. 1952, Catlett and Millich 1976). Thrombocyte counts in our study were lower than those (51,550 ± 3,323 cells/mm^3) reported by Watson et al. (1963). The 95-percent confidence limits overlap between the value given by Watson et al. (1963) and that reported for group 2 in our study. Although total counts for leucocytes and lymphocytes in our study were about half of those reported by Watson et al. (1963), the confidence limits nearly overlap for total leucocyte counts, and the confidence limits for lymphocyte counts overlap between the counts reported for group 2 in our study and those reported by Watson et al. (1963). Moreover, the percentage of lymphocytes to total leucocytes for group 1 (94.5 percent) and group 2 (92.9 percent) compare favorably with the values (92.5 percent) obtained by Watson et al. (1963). Values for other leucocytic cells, which were quite variable, were less than those reported by Watson et al. (1963).

Some authors have suggested that there is a relationship between fish size or sex and erythrocyte counts (Haws and Goodnight 1963, Houston and DeWilde 1969, DeWilde and Houston 1967). In some studies, fish size appeared to be directly correlated with erythrocyte counts (Haws and Goodnight 1962, Houston and DeWilde 1969), whereas in other studies fish size appeared to be inversely correlated with erythrocyte counts (DeWilde and Houston 1967). Other work showed no apparent correlations between either fish size or sex and erythrocyte counts (McKnight 1966). We found no apparent size- or sex-related variations in erythrocyte counts in goldfish.

Leucocyte-thrombocyte differential counts, which represent the quantitative white blood cell distributions, are given in Table 10. Thrombocytes were the dominant white blood cell type accounting for approximately 80 percent. Lymphocytes and neutrophils accounted for about 10 percent and 8 percent, respectively. The normal distributions of eosinophils and hemoblasts were observed to be less than 1 percent. Although basophils were observed for the volumetric determination, none were observed in the blood smear analyses. Macrophages were also not observed.

Significant differences in the distribution of thrombocytes, neutrophils and eosinophils were associated with differences in culture density. With respect to the controls, the distribution of thrombocytes decreased by about 9 percent, whereas the neutrophil count doubled and the distribution of eosinophils increased nine-fold in the high density culture.

Cellular distribution determined by cell differentiation from dry smears stained with Wright's stain was somewhat different than that determined by volumetric counts of cells diluted and stained with Shaw's solution. Volumetric counts had higher percentages of lymphocytes and lower percentages of other leucocytic cell types than distributions determined by counts on dry smears. The differences in these results may be associated with differences in techniques for preparing and assaying wet mounts vs. dry mounts. The Wright's stain may be a better stain for differentiating between leucocytic cell types. It is also possible that there may be more mechanical stress on the cells of dry smears, thereby resulting in tearing of the cells. However, relatively few torn cells were found in this study. Counts from dry mounts are routinely used to determine cell distributions because of the higher precision.

Although our volumetric counts agreed with those previously reported for goldfish (Watson et al. 1963), our differentials (from blood smears) were at variance with those reported by Bennett and Neville 1975. This variance may be accounted for by differences in fish culture conditions, including holding density, acclimation period, feeding, fish size, photoperiod and temperature.

Distribution of leucocytes may be a useful indicator of unfavorable environmental conditions or stress. Lymphopenia has been observed in juvenile coho salmon acutely stressed by crowding and elevated temperature (McLeay 1975). Both lymphopenia and neutrophilia were observed in juvenile pink salmon held under crowded and hypoxic conditions (Belova in Conroy 1972). Since no change was observed in lymphocyte distribution in our study, lymphopenia may have resulted in response to acute stresses of hypoxia, elevated temperature shock, or either of these stresses in combination with crowding. Although neutrophilia was attributed to hypoxia (Belova in Conroy 1972), it was also observed in crowded goldfish. While the crowding syndrome has not yet been established, these studies support the inclusion of neutrophilia. The present study suggests that thrombopenia and eosinophilia may further characterize the leucocyte distribution associated with crowding.

Tables 11 and 12 summarize possible effects of density on the morphology of red blood cells. Cell morphology was virtually the same among fish within each group, indicating possible density-dependent responses (Table 11). Significant morphological differences were observed between the two groups (Table 12). Smaller, normal-shaped cells were found in the fish in group 2: the length, width, and area of the cells in group 2 were 0.3, 0.7, and 1.07 percent less, respectively, than those in the fish in group 1.

The values obtained for erythrocyte morphology agree with values reported by other authors. Our values for mean cell length and width are well within the ranges reported for goldfish by Smith et al. (1952) and Watson et al. (1963). Because we found no studies on goldfish that reported values for roundness and area, we calculated these values (Table 13) from reported values of erythrocyte length and width for goldfish (Watson et al. 1963, Smith et al. 1952) and some other teleosts (Haws and Goodnight 1962). Comparison of these calculated values with values obtained in our study showed our values to be higher than those obtained by Watson et al. (1963), but lower than those obtained by Smith et al. (1952). Similarly, the area values found in our study were greater than those found for channel catfish, but less than those for brown bullhead. Cell roundness values in our study were less than those found for either channel catfish or brown bullhead by Haws and Goodnight (1962).

Fish red blood cell morphology is sensitive to a variety of environmental stresses (Haws and Goodnight 1962, Conroy 1970; McCarthy et al. 1973). In this study, we identified loading density as an important environmental stress, as evidenced by changes in blood cellular distribution and erythrocyte morphology. Changes associated with high density include a reduced number of smaller but normal-shaped red blood cells, thrombopenia, neutrophilia, and eosinophilia.

II. Catfish Studies

The effects of intensive culture practices of catfish on red blood cell morphology are summarized in Table 14. These results indicate three distinct groupings of the data for cell length, width, and area, viz. the control density (0.18 kg/m^3), medium density (224 kg/m^3), and high density (337 kg/m^3 and 433 kg/m^3). Results for cell roundness indicate two distinct groups where data from control and medium densities comprised one group, and the data from both densities comprised the other group. Medium and high loading densities tended to decrease cell length by 7.8 percent and 14.6 percent, cell width by 6.3 percent and 9.1 percent, cell area by 13.1 percent and 21.8 percent, and high density tended to increase cell roundness by about 5 percent.

The biological signficance of red blood cell morphology is at least two-fold: (1) cell size determines the available volume for oxygen transport, gaseous exchange, and transport of nutrients and biochemical products, whereas (2) cell shape, which influences the surface to volume ratios, determines, in part, the rates of cellular exchanges. The cell morphology associated with fish cultured under normal density conditions is assumed to be optimum for red blood cell functions. Since cell morphology and physiology are closely allied, deviations from the normal morphology may indicate physiological perturbations. If a density syndrome were revealed by red blood cell morphology, then it should be possible to identify and isolate the effects of density stress from a physiological stress resulting from a toxicant.

Since only one study has reported the effects of density on red blood cell morphology, the comparable data base is meager. Nevertheless, the results of this study reinforce those observed for goldfish (Murray and Burton 1978). In both studies red blood cells from fish in the control group had longer, wider cells, with more area than did those cells from fish cultured at higher loading densities. The only apparent difference between the two studies is in the cell shape (roundness). In the goldfish study there was no significant difference between the control group and the high density group. In the present study cell shape increased significantly at the high densities. Results in Table 14 indicate no difference, however, between the control group and the medium density group. Since the medium density group for the present study had a higher density than the high density in the goldfish study, the conclusion is similar for both studies, viz, cell shape changes only under prolonged extreme stress conditions.

While these changes are highly significant, it was desirable to see if better statistical resolution could be obtained with more than 50 cellular measurements. A comparison of 50 with 250 cellular measurements was made. These results are shown in Table 15. The mean values were significantly reduced by 250 measurements as compared to 50 measurements, 3.5 percent for cell length and width, and 6.7 percent for cell area. The mean values for cell roundness were similar for both groups. As expected, the coefficients of variation for the 250 measurement group were consistently less than those for the 50 measurement group, indicating better resolution of the mean.

When this study began, the proper number of cellular measurements was not known. This number was determined empirically in the goldfish study to be approximately 200 cell measurements for best statistical characterization of the cell features. Results from this study indicate that fewer measurements can be used with good results provided that the statistical model used is sensitive enough, such as hierarchical designs.

Results from this study support those observed in the goldfish study. Smaller and rounder red blood cells were consistently found in fish loaded at high densities. Evidently, in fish a density stress syndrome exists for red blood cell morphology.

III. Culture Complexity Studies

The bluegill in the monoculture (group 1) were longer than those in the other groups (Table 17). No significant correlations were observed, however, between length and any of the hematological variables. Hematological data are summarized by group in Table 17.

When all the data were analyzed together, there was a marked effect of culture composition on adult bluegill hematology (Table 18, 5 groups). With respect to the controls (group 1) culturally stressed bluegill (groups 2-5) showed erythrocytosis, reduced mean cell hemoglobin values, more elliptically shaped red blood cells with reduced cell area, and neutrophilia (Tables 17 and 19). The other differences noted in Table 19 were not well-defined. Overall, the best hematological indicators for culture stress appear to be cell morphology (cell area, width and length), hemoglobin and hematocrit values. The fact that ten variables accounted for only 84.5 percent of the total variation (Table 20) not only indicates the complexity of the hematological responses but also the nonspecificity of the response to culture stress. Results of partial correlation analysis were not unusual (Table 21). When the data are examined for a particular culture stress, the results, discussed below, appear to be more specific.

Results of multivariate analysis of variance of the data for groups 1 and 2 indicate that the use of monoculture data for controls or use in bioassays is apparently valid (Table 18, groups 1 and 2). Analysis of the data by univariate analysis of variance indicates differences between the two groups for five hematological variables (Table 19). However, these variables accounted for only 63.77 percent of the total variation (Table 22). Results of the partial correlation analysis were not unusual (Table 23).

Density stress by a second species was significant (Table 18, groups 2, 3, and 4). Nine hematological variables accounted for at least 80 percent of the variance, but only five of them attributed to differences between the groups (Tables 19 and 24). Observed differences between the lower density groups (groups 2 and 3) and the high density groups (group 3) ranged from a 19-20 percent decrease in hemoglobin levels, a 15-20 percent decrease in hematocrit values, and an 8-11 percent decrease in erythrocyte length. No apparent differences were observed between the low and medium density groups (groups 2 and

3) for hemoglobin, hematocrit and erythrocyte length. Nonlinear responses were observed for erythrocyte width and erythrocyte area, where (1) cell area was similar for both the low and high density groups and (2) cell width increased by 6-7 percent and cell area increased by 10-15 percent in the medium density group. The albumin/protein ratios were significantly higher (5-fold) for the medium and high density groups with respect to the low density group. The hematological correlations for density stress, indicated in Table 25, were not unusual.

A density stress syndrome has been identified in intensive monocultures of catfish and goldfish at the cellular level of blood, where smaller and rounder cells were observed for the high loading densities (Murray and Burton 1979, Murray 1980). Blood cells of bluegill subjected to high densities responded similarly. Based on the results of this study and those of the goldfish study (Burton and Murray 1979) the density stress syndrome may be extended to include decreased hematocrit and hemoglobin values with a possible concomitant increase in albumin/protein ratio.

The data indicate that bluegill in complex cultures, consisting of 3 fish species, were stressed as measured by hematological changes (Table 18, groups 1 vs 5). Six hematological variables contributed to the differences between the control and triculture groups (Table 19). These variables accounted for 84.19 percent of the variance (Table 26). It is noteworthy that cell area and cell width variables each contributed at least 20 percent to the variance (Table 26). Morphological variables, as well as cellular abundance and packed cell volume appear to be good indicators of stress induced by complex cultures. Examination of the data shows large erythrocytes in the complex culture (Table 17). With respect to the monoculture cell area was greater by 16.58 percent, cell width by 10.07 percent and cell length by 5.86 percent. The abundance of red blood cells was also greater in the complex culture by 29.47 percent as was the hematocrit by 28.38 percent. The decreased value for MEHc was also significant. None of the hematological correlations was unusual (Table 27). However, numerous relationships that were observed previously were not significant for the complex culture stress.

In the triculture stress situation some blood dyscrasias, which are possibly gnomonic, included reduced MEH, elevated hematocrit and erythrocytosis. One would suspect hemoconcentration with these conditions. Hemoconcentration was not indicated, however, since plasma osmolality remained constant. Furthermore, respiratory function was apparently not impeded as evidenced by the hemoglobin values. Therefore, erythrocytosis must have compensated for the reduced oxygen-carrying capacity per cell.

This study indicates the need to identify and control the culture conditions for baseline and bioassay surveys.

IV. Bluegill Hematology

Although an attempt was made to work with fish of uniform size throughout the study, fish sampled in June 1978 were significantly longer than the fish sampled in August, November, or February (Table 28). The data also indicate that males were approximately 4 percent longer than females. Since there were no significant correlations between body length (L) and any of the hematological parameters, these variations in length were not considered to impact the hematological findings in this study.

A. Blood Chemistry

1. Hemoglobin (HB). Hemoglobin levels were fairly constant in November, February, and June but decreased approximately 10 percent in August (Table 28). Differences attributable to sex were found for hemoglobin values, where values for males were 9 percent less than those for females. No significant interactions were observed between season and sex.

2. Hematocrit (HC). Values for hematocrits were virtually constant during June, August, and November, increasing by 7-11 percent during February (Table 28). Although no differences attributable to sex were observed, there were significant seasonal-sex interactions where hematocrit values for females increased 5-12 percent during November and February and values for males increased 9-18 percent during August and February.

3. Erythrocytic Sedimentation Rate (ESR). No seasonal or sex differences were observed for ESR values (Table 28). However, the very low ESR values during August and the very high values during November for males are noteworthy.

4. Protein (P). Protein levels were considerably decreased (by 25-35 percent) during February compared to other times of the year (Table 28). There were no apparent differences between sexes in protein levels. In addition, no significant seasonal-sex interactions were found.

B. Hematological Indices

1. Erythrocyte abundance (RBC). Erythrocyte abundance was approximately 11-17 percent lower in February and June than in August and November (Table 29). Males had approximately 10 percent fewer erythrocytes than females. There were, however, no significant seasonal-sex interactions.

2. Mean Erythrocytic Volume (MEV). Values for MEV were 11-32 percent higher in February and June than those for August and November (Table 29). Elevated values (by 9 percent) were also observed for males, although no significant seasonal-sex interactions were found.

3. Mean Erythrocytic Hemoglobin (MEH). Values for MEH were about 21-28 percent higher in February and June than those for August and November (Table 29). However, there were no significant differences between values for males and females, nor were there any significant seasonal-sex interactions.

4. Mean Erythrocytic Hemoglobin Concentration (MEHc). The MEHc values remained fairly stable during the year. However, females had approximately 7 percent higher values for MEHc than males. On the other hand, there were no significant seasonal-sex interactions (Table 29).

C. Leucocyte-Thrombocyte Differentials

1. Thrombocytes (THR). Thrombocytes were the most dominant type of white blood cell, comprising 80-90 percent (Table 30) of the total. The distribution of thrombocytes was 8-10 percent less during February and June than during August and November. Thrombocyte abundance was similar in males and females, ranging from 85-86 percent.

2. Lymphocytes (LYM). Lymphocytes were the most abundant leucocyte present, comprising 7-13 percent of the white blood cells (Table 30). The distribution of lymphocytes was similar during August and November, ranging from 8-8.5 percent. Concomitant with the reduction of thrombocytes in February and June, the lymphocytes increased by 2 percent in February and 5 percent in June. Similar to the thrombocytes, no differences between sexes nor seasonal-sex interactions were observed in the lymphocyte values.

3. Neutrophils (NEU). Neutrophil distribution comprised approximately 4-9 percent of the white blood cells (Table 30). When compared with other times of the year, neutrophil abundance increased by about 3-5 percent during February (Table 30). Although no significant variations in neutrophil abundance were associated with fish sex alone, significant seasonal-sex interactions were found. Neutrophil distribution is apparently more variable in males than females, where in the males low values were observed in August and June, high values in February. Neutrophil counts were fairly constant each month for females.

4. Eosinophils (EOS). Approximately 0.1-0.5 percent of the leucocyte-thrombocyte differentials were eosinophils (Table 30). The percentage of eosinophils present in the blood of bluegill remained relatively stable during the months examined in this study. It was observed, however, that females had twice as many eosinophils as males.

5. Basophils (BAS). Very few basophils (up to 0.1 percent) were observed in bluegill blood of the present study (Table 30). The percentage of basophils remained consistently low, with no significant differences between sexes or seasons.

6. Hemoblasts (HEM). Hemoblasts also represented a small portion of the leucocyte-thrombocyte differentials, comprising about 0.1-0.2 percent of the total count (Table 30). Hemoblast abundance remained unchanged regardless of season and/or sex.

D. Red Blood Cell Morphology

1. Cell Length (CL). The red blood cells of bluegill ranged from approximately 10-30 µm in length (Table 31). Similar values for cell length were observed in fish sampled during November and February. Compared with these values, cells were 8-9 percent shorter during August and 3-5 percent larger during June. Similar cell length values were found for males and females, averaging around 12 µm.

2. Cell Width (CW). The average cell width for males and females was about 8 µm (Table 31). Although the widths ranged from 7-9.5 µm throughout the study, the lowest values were observed during November, the highest values during June, and intermediate values during August and February.

3. Cell Height (CH). The cell height of bluegill erythrocytes varied from approximately 3-5 µm (Table 31). Erythrocytic height was similar during November and June, but was about 14-25 percent more than that during February and August where the values were around 4-4.5 µm. Similar values were also observed in males and females.

4. Cell Roundness (CR). Bluegill erythrocytes are ellipsoid in shape. During November and February the ellipsoid shapes were similar (Table 31). Rounder cells were observed for August (by 11-13 percent) and June (by 14-16 percent). Apparently rounder cells were associated with warmer temperatures.

5. Cell Area (CA). On the average the cell area ranged from 72.5-73.5 μm^2 (Table 31). The cell area appeared to increase from August to November to February to June. During the year the total increase in cell area was approximately 34 percent, with the largest increase (23.9 percent) occurring between February and June.

E. Hematological Relationships. The hematological relationships were determined from the results of partial correlation analysis (Table 32). With the exception of the cell height and mean erythrocytic hemoglobin, no relationships were unusual. Hemoglobin was positively associated with mean erythrocytic hemoglobin and mean erythrocytic hemoglobin concentration. Hematocrit was positively associated with red blood cell abundance, mean erythrocytic volume, and cell height, and inversely related to erythrocytic sedimentation rate and mean erythrocytic hemoglobin concentration. Erythrocytic sedimentation rate was additionally related with mean erythrocytic hemoglobin. Red blood cell abundance was inversely related with mean erythrocytic volume and mean erythrocytic hemoglobin because it is used in calculating these hematological indices. In addition, red blood cell abundance was inversely associated with cell height. Apparently thick corpuscles have a limiting effect on red blood cell abundance. Mean erythrocytic volume was directly related to cell height because it was used to calculate cell height. Mean erythrocytic hemoglobin and mean erythrocytic hemoglobin concentration were positively correlated. Cell morphology parameters were closely associated with each other. Cell length was directly associated with cell width and cell area and inversely associated with cell roundness and cell height. Cell width was directly related to cell roundness and cell area, and cell area was directly associated with cell height. Thrombocyte distribution was inversely related with the distribution of lymphocytes and neutrophils.

F. Sex-Seasonal Influence. Influences of sex and/or season on bluegill hematology are summarized in Table 33. Results of the multivariate analysis of variance indicate hematological variances with sex, season, and the sex-season interaction. The univariate tests (Tables 28-31) indicate significant changes in red blood cell numbers, hemoglobin levels, mean erythrocytic volume, and mean erythrocytic hemoglobin concentrations between the sexes. More changes were observed due to seasonal differences, viz.: red blood cell width, area, roundness and height, distribution of thrombocytes, lymphocytes, neutrophils, and eosinophils, MEV, MEH, RBC, and HC. The seasonal-sex interactions were influenced primarily by changes in cell width, length and area, hematocrit and erythrocytic sedimentation rate, and distribution of neutrophils.

The percentage of the variance that each variable contributed to the total variance is indicated in Table 34. Three variables each contributed more than 10 percent to the total variance, seven variables each contributed more than 4 percent, while eight variables contributed less than 4 percent each to the total variance. Ten variables accounted for more than 80 percent of the variance. Variables related with red blood cell morphology and distributon of thrombocyte-leucocytes accounted for most of the variance.

G. Baseline Results. Little has been documented on the hematology of bluegills. Some blood parameters are listed in Table 35 for comparison with results from this study. In general there is fairly good agreement of results considering the number of fish examined. In this study, 160 fish were examined, compared to 12 by Burton (1979) and 27 by Smith et al. (1952), and 1 by Smith et al. (1952) for cell morphology. The values for hematocrit vary by 5 percent. Variations in the hemoglobin values may be due to methodological differences. Red blood cell counts vary by approximately 4 percent, which is well within the expected variability in such counts. The variation in the mean erythrocytic hemoglobin is due to methodological differences in hemoglobin determinations as well as variations in red blood cell counts. Cell length and width measurements were smaller in the study by Smith et al. (1952). However, only 10 cells were measured in one fish. In the present study, 200 cells were measured for each of the 160 fish examined.

The purpose of this study was to document and broaden the data base on bluegill hematology taken from normal and stressed animals. Tests were also designed to determine overall effects of season and/or sex on bluegill hematology. The study of fish blood is complicated by sex and/or seasonal differences, as documented in this report. In addition, the selection of hematological indicators is somewhat difficult since 10 blood parameters accounted for approximately 80 percent of the total variance. In general, good hematological indicators appear to be those associated primarily with cell morphology and secondarily with thrombocyte-leucocyte distribution.

TABLE 1. BLOOD CHEMISTRY OF SOME FRESHWATER TELEOSTS

Species	Common Name	Osmolality (mosm/1)	Total Protein (g/100ml)	Albumin (g %)	Albumin Total Protein (%)	Glucose (mg/100 ml)	Hemoglobin (g/100ml)	Reference
Salvelinus fontinalis	Brook Trout					68	8.5	Houston *et al*. 1971
Salvelinus fontinalis	Brook Trout		3.5[2]	2.10	60.7	70[1]	8.5[2]	Field *et al*. 1943
Salmo trutta	Brown Trout						6.8	Blaxhall & Daisley 1973
Salmo gairdneri	Rainbow Trout						7.3[2]	Houston & Cyr 1974
Salmo gairdneri	Rainbow Trout		5.0			128	7.5	McCarthy *et al*. 1973
Salmo gairdneri	Rainbow Trout	258						Rao 1969
Salmo gairdneri	Rainbow Trout		3.5[2]			70		Schreck *et al*. 1976
Salmo gairdneri	Rainbow Trout		8.0			202	9.3	Barnhart 1969
Prosopium williamsoni	Mountain Whitefish						10.6	McKnight 1966
Cyprinus carpio	Carp		4.2[2]	2.82	68.0	111[1]	10.5[2]	Field *et al*. 1943
Carassius auratus	Goldfish	264					10.6	Burton & Murray 1979
Carassius auratus	Goldfish	295	4.4			42		Catlett & Millich 1976
Carassius auratus	Goldfish						36.0[3]	Falkner & Houston 1966
Carassius auratus	Goldfish						7.6	Houston & Cyr 1974
Carassius auratus	Goldfish		1.9				8.3	Summerfelt *et al*. 1967
Notemigonus crysoleucas	Golden Shiner		7.4				7.5	Courtois 1975a
Notemigonus crysoleucas	Golden Shiner		6.4				7.4	Courtois 1975a
Fundulus diaphanus	Banded Killifish	375						Ahokas & Sorg 1977
Fundulus heteroclitus	Mummichog	372						Umminger 1970b
Fundulus heteroclitus	Mummichog					50[1]		Umminger 1971
Ictiobus cyprinellus	Bigmouth Buffalo						7.8	Chlebeck & Phillips 1969
Ictiobus bubalus	Smallmouth Buffalo						8.5	Chlebeck & Phillips 1969
Ictalurus punctatus	Channel Catfish	275	2.9[2]			58[1]		Block 1974
Ictalurus punctatus	Channel Catfish						6.6	Haws & Goodnight 1962
Ictalurus nebulosus	Brown Bullhead						6.9	Haws & Goodnight 1962
Lepomis macrochirus	Bluegill						242.0[4]	Graham 1974
Morone saxatilis	Striped Bass		7.8				8.9	Courtois 1976
Labeo umbratus	Mudfish	262	30.3				7.2[2]	Hattingh & Van Pletzen 1974

1 assuming mg % = mg/100ml
2 assuming g % = g/100ml
3 total blood iron level in mg/100g
4 plasma iron level in ug%

TABLE 2. BLOOD PHYSIOLOGY OF SOME FRESHWATER TELEOSTS

Species	Common Name	Hc (%)	ESR (mm/hr)	MEV (u^3)	MEH (uug)	MEHC (%)	RBC Count (millions per mm^3)	WBC-T Count (thousands per mm^3)	Reference
Alosa pseudoharengus	Alewife	55							Stanley & Colby 1971
Salvelinus fontinalis	Brook Trout	27		314	75		1.01	3.9[2]	Field *et al.* 1943
Salvelinus fontinalis	Brook Trout	32							Houston *et al.* 1971
Salvelinus fontinalis	Brook Trout	51							Snieszko 1961
Salmo trutta	Brown Trout	32	1.2				1.00	11.5[2]	Blaxhall & Daisley 1973
Salmo trutta	Brown Trout	40							Snieszko 1961
Salmo gairdneri	Rainbow Trout	43							Houston & Cyr 1974
Salmo gairdneri	Rainbow Trout	40	1.7	380	73	19	1.07	14.0	McCarthy *et al.* 1973
Salmo gairdneri	Rainbow Trout	48							Snieszko 1961
Salmo gairdneri	Rainbow Trout	23							Weber *et al.* 1976
Salmo gairdneri	Rainbow Trout	35	1.9				1.45		Barnhart 1969
Oncorhynchus kisutch	Coho Salmon	39							Giles & Vanstone 1976
Oncorhynchus kisutch	Coho Salmon						0.91	56.4	McLeay 1975
Salvelinus namaycush	Lake Trout	30					0.80		Piper & Stephens 1962
Prosopium williamsoni	Mountain Whitefish	44	1.2				1.57	23.0	McKnight 1966
Cyprinus carpio	Carp				55	42	1.31		Smith *et al.* 1952
Cyprinus carpio	Carp	31		311	72		0.84	3.7[2]	Field *et al.* 1943
Carassius auratus	Goldfish						1.80	56.2	Murray & Burton 1979
Carassius auratus	Goldfish	35		185			1.88		Catlett & Millich 1976
Carassius auratus	Goldfish	29		165		17[1]	2.15		Falkner & Houston 1966
Carassius auratus	Goldfish	40	2.0	241	63	26			Burton & Murray 1979
Carassius auratus	Goldfish	35							Houston & Cyr 1974
Carassius auratus	Goldfish				41	30	1.24		Smith *et al.* 1952
Carassius auratus	Goldfish	31							Summerfelt *et al.* 1967
Carassius auratus	Goldfish						2.02	73.6	Watson *et al.* 1963
Notemigonus crysoleucas	Golden Shiner	40							Courtois 1975a
Notemigonus crysoleucas	Golden Shiner	42							Courtois 1975a
Ictiobus cyprinellus	Bigmouth Buffalo	35			67		1.08	23.9	Chlebeck & Phillips 1969
Ictiobus bubalus	Smallmouth Buffalo	33			64		1.28	29.0	Chlebeck & Phillips 1969
Ictiobus bubalus	Smallmouth Buffalo				52	40	1.33		Smith *et al.* 1952
Minytrema melanops	Spotted Sucker				84	61	1.22		Smith *et al.* 1952
Ictalurus punctatus	Channel Catfish	40							Block 1974
Ictalurus punctatus	Channel Catfish	29					2.16		Haws & Goodnight 1962
Ictalurus nebulosus	Brown Bullhead	28					1.22		Haws & Goodnight 1962
Ameiurus melas	Black Bullhead				58	56	1.61		Smith *et al.* 1952
Ameiurus natalis	Yellow Bullhead				62	56	1.49		Smith *et al.* 1952
Lepomis macrochirus	Bluegill				29	45	2.60		Smith *et al.* 1952
Chaenobryttus coronarius	Warmouth				34	38	1.88		Smith *et al.* 1952
Pomoxis annularis	White Crappie				47	49	1.80		Smith *et al.* 1952
Morone saxatilis	Striped Bass	57							Courtois 1976
Labeo umbratus	Mudfish	26					1.87		Hattingh & Van Pletzen 1974

[1] mean erythrocytic iron concentration in mg x 10^{-11}

[2] count does not include thrombocytes

TABLE 3. ERYTHROCYTE MORPHOLOGY OF SOME FRESHWATER TELEOSTS

Species	Common Name	Length (u)	Width (u)	Roundness[1]	Area[1] (u^2)	Reference
Salmo trutta	Brown Trout	15.2	9.0	0.59	107.4	Blaxhall & Daisley 1973
Salmo gairdneri	Rainbow Trout	15.7	10.4	0.66	128.2	McCarthy et al. 1973
Cristivomer namaycush	Lake Trout	17.8	10.4	0.58	145.4	Lieb et al. 1953
Thymallus signifer	American Grayling	13.2	9.5	0.72	98.5	Lieb et al. 1953
Prosopium williamsoni	Mountain Whitefish	13.6	9.3	0.68	99.3	McKnight 1966
Cyprinus carpio	Carp	13.4	10.2	0.76	107.4	Smith et al. 1952
Carrassius auratus	Goldfish	13.7	8.5	0.62[2]	92.3[2]	Murray & Burton 1979
Carrassius auratus	Goldfish	13.4	8.8	0.66	92.6	Watson et al. 1963
Carrassius auratus	Goldfish	13.3	8.0	0.60	83.6	Smith et al. 1952
Ictiobus bubalus	Smallmouth Buffalo	15.3	9.0	0.59	108.2	Smith et al. 1952
Minytrema melanops	Spotted Sucker	14.0	9.1	0.65	100.1	Smith et al. 1952
Ictalurus punctatus	Channel Catfish	11.0	7.6	0.69	65.5	Haws & Goodnight 1962
Ictalurus nebulosus	Brown Bullhead	13.8	9.4	0.68	102.7	Haws & Goodnight 1962
Ameiurus natalis	Yellow Bullhead	12.4	10.5	0.85	102.3	Smith et al. 1952
Lepomis macrochirus	Bluegill	10.9	6.4	0.59	54.8	Smith et al. 1952
Chaenobryttus coronarius	Warmouth	11.1	7.7	0.69	67.1	Smith et al. 1952
Pomoxis annularis	White Crappie	11.8	7.2	0.61	66.7	Smith et al. 1952
Micropterus salmoides	Largemouth Bass	12.1	6.8	0.56	64.6	Smith et al. 1952

1 calculated from reference except where noted
2 given in reference

TABLE 4. CELLULAR DISTRIBUTION OF THE BLOOD OF SOME FRESHWATER TELEOSTS[1]

Species	Common Name	Thrombocytes (%)	Leucocytes (%)	Lymphocytes (%)	Granulocytes (%)	Other Cell Types	Reference
Salmo trutta	Brown Trout	-[2]	100.0	90.0	9.6	0.3	Blaxhall & Daisley 1973
Salmo gairdneri	Rainbow Trout	1.7	98.3	89.2	9.1	0.0	Schreck *et al.* 1976
Salmo gairdneri	Rainbow Trout	1.8	98.2	93.5	4.8	0.0	McCarthy *et al.* 1973
Cristivomer namaycush	Lake Trout	-[2]	100.0	91.2	8.8	0.0	Lieb *et al.* 1953
Prosopium williamsoni	Mountain Whitefish	-[2]	100.0	33.9	66.1	0.0	McKnight 1966
Carassius auratus	Goldfish	-[2]	100.0	80.4	19.6	0.0	Bennett & Neville 1975
Carassius auratus	Goldfish	82.0[3]	18.0[3]	17.0[3]	1.0[3]	0.0[3]	Murray & Burton 1979
Carassius auratus	Goldfish	-[2]	100.0[3]	92.5[3]	7.5[3]	0.0[3]	Watson *et al.* 1963

[1] classifications based on the categories of Watson et al. 1963

[2] thrombocytes not reported

[3] determined by volumetric counts

TABLE 5. HEMATOLOGY VALUES FOR GOLDFISH, *Carassius auratus L.*, AT TWO DIFFERENT DENSITIES.

Characteristic	Group 1[a] (Mean ± SE)	Group 2[b] (Mean ± SE)	t[c]
Hc, %	40.22 ± 1.13	38.00 ± 1.04	**
Hb, g/100 ml	10.60 ± 0.23	9.70 ± 0.26	**
ESR, %	2.03 ± 0.18	7.10 ± 1.29	**
MEV, cμ	241 ± 10	245 ± 11	NS
MEH, μμg	63 ± 3	66 ± 3	NS
MEHc, %	26 ± 0.6	26 ± 0.4	NS
PO, mOsm/1	263.6 ± 2.24	262.9 ± 1.92	NS
Length, cm	19.65 ± 0.20	26.00 ± 0.41	**
Glucose, mg/100 ml	69.67 ± [illegible]	90.62 ± 7.50	*
Total protein, g/100 ml	3.39 ± 0.07	3.54 ± 0.14	NS
Albumin, mg/100 ml	0.204 ± 0.029	0.366 ± 0.074	NS
Albumin/total protein, %	0.0065 ± 0.0010	0.0118 ± 0.0022	*

a. 60 fish per 400-gallon tank
b. 240 fish per 400-gallon tank
c. Symbols: *--P≥ 0.05; **--P≥ 0.01; NS--not significant

TABLE 6. SUMMARY OF RESULTS FOR CORRELATION COEFFICIENT BETWEEN LENGTH AND SELECTED BLOOD CHARACTERISTICS.[a]

Group	L-Hc	L-Hb	L-ESR
Combined densities	**	**	**
Male	NS	NS	*
Female	NS	NS	**
Group 1	NS	**	NS
Male	NS	NS	NS
Female	NS	*	NS
Group 2	NS	NS	NS
Male	NS	NS	NS
Female	NS	NS	NS

a. Symbols: *--$P \geq 0.05$; **--$P \geq 0.01$; NS--not significant

TABLE 7. COMPARISONS OF SELECTED BLOOD CHARACTERISTICS BETWEEN SEXES FOR COMBINED AND INDIVIDUAL DENSITY GROUPS.

Characteristic	Group	Males (Mean ± SE)	Females (Mean ± SE)	t[a]
HC, %	Combined densities	40.67 ± 0.75	36.19 ± 1.56	**
	Group 1	41.97 ± 0.88	37.38 ± 2.61	*
	Group 2	39.12 ± 1.24	34.90 ± 1.63	NS
HB, g/100 ml	Combined densities	10.47 ± 0.20	9.35 ± 0.32	**
	Group 1	10.99 ± 0.22	9.41 ± 0.56	**
	Group 2	9.88 ± 0.33	9.30 ± 0.34	NS
ESR, %	Combined densities	3.15 ± 0.63	6.89 ± 1.45	**
	Group 1	1.66 ± 0.15	2.60 ± 0.55	*
	Group 2	5.18 ± 1.42	11.52 ± 2.38	*
Length, cm	Combined densities	22.02 ± 0.39	24.77 ± 0.80	**
	Group 1	19.30 ± 0.17	20.69 ± 0.52	**
	Group 2	25.08 ± 0.42	27.94 ± 0.76	**

a. Symbols: *$P \geq 0.05$; **--$P \geq 0.01$; NS--not significant

TABLE 8. COMPARISONS OF SELECTED BLOOD CHARACTERISTICS BETWEEN TWO LOADING DENSITIES FOR EACH SEX.

Characteristic	Sex	Group 1 (Mean ± SE)	Group 2 (Mean ± SE)	t[a]
Hc, %	Male	41.97 ± 0.88	39.12 ± 1.24	NS
	Female	37.38 ± 2.61	34.90 ± 1.66	NS
Hb, g/100 ml	Male	10.99 ± 0.22	9.88 ± 0.33	**
	Female	9.41 ± 0.56	9.30 ± 0.34	NS
ESR, %	Male	1.66 ± 0.15	5.18 ± 1.42	**
	Female	2.60 ± 0.55	11.52 ± 2.38	**
Length, cm	Male	19.30 ± 0.17	25.08 ± 0.42	**
	Female	20.69 ± 0.52	27.94 ± 0.76	**

a. Symbols: *--$P \geq 0.05$; **--$P \geq 0.01$; NS--not significant

TABLE 9. NUMBER OF CELLS PER CUBIC METER IN Carrasius auratus L. AT TWO LOADING DENSITIES.

Cell Type	Group 1[a] (Mean ± SE)	Group 2[b] (Mean ± SE)	t	df	Significance[c]
Erythrocytes	$1.80 \pm 0.06 \times 10^{6}$	$1.60 \pm 0.07 \times 10^{6}$	2.18	110	*
Thrombocytes	$4.61 \pm 0.36 \times 10^{4}$	$3.00 \pm 0.22 \times 10^{4}$	3.73	108	**
Leucocytes	$1.01 \pm 0.07 \times 10^{4}$	$1.47 \pm 0.19 \times 10^{4}$	2.23	108	*
Lymphocytes	$9.54 \pm 0.70 \times 10^{3}$	$13.66 \pm 1.91 \times 10^{3}$	2.08	108	*
Other	$5.53 \pm 1.46 \times 10^{2}$	$10.37 \pm 2.42 \times 10^{2}$	1.74	108	NS
Leucocytes/rbc	0.0059 ± 0.0004	0.0091 ± 0.0010	3.06	108	**

a. 60 fish per 400-gallon tank.
b. 240 fish per 400-gallon tank.
c. Symbols: *--$P \geq 0.05$; **--$P \geq 0.01$; NS--not significant.

TABLE 10. SUMMARY OF THE RESULTS OF THE STUDENT'S T-TEST BETWEEN TREATMENT GROUPS FOR EACH CELL TYPE EXAMINED IN LEUCOCYTE-THROMBOCYTE DIFFERENTIAL COUNTS.[1]

Cell Type	Density I (Mean ± SE) (%)	Density II (Mean ± SE) (%)	df	t	Significance
Thrombocytes	82.46 ± 0.67	74.94 ± 1.19	108	5.60	**
Lymphocytes	9.70 ± 0.36	9.28 ± 0.40	108	0.77	NS
Neutrophils	7.72 ± 0.66	15.53 ± 1.13	108	6.06	**
Eosoinophils	0.01 ± 0.01	0.09 ± 0.04	108	2.25	*
Hemoblasts	0.11 ± 0.03	0.22 ± 0.05	108	1.75	NS

1 = No basophils or macrophages were present
NS = Not significantly different from control
* = Significantly different from controls, $P>0.05$
** = Significantly different from controls, $P\geq0.01$

TABLE 11. SUMMARY OF ANALYSIS OF VARIANCE TESTS FOR BLOOD CELL MORPHOLOGY OF *Carrasius aratus* L. AT TWO LOADING DENSITIES WITH TWO HUNDRED CELL MEASUREMENTS FOR EACH OF THE 111 FISH.

Measurement	VS[a]	df	MS	F[b]
Length	Among fish	109	26.37	0.36 NS
	Between groups	1	8.10	17.47 **
	Error	22,089	0.46	
Width	Among fish	109	16.94	1.31 NS
	Between groups	1	22.16	54.20 **
	Error	22,089	0.41	
Roundness	Among Fish	109	0.1054	0.06 NS
	Between groups	1	0.0062	2.20 NS
	Error	22,089	0.0028	
Area	Among fish	109	4,196.10	1.30 NS
	Between groups	1	5,437.30	64.21 **
	Error	22,089	84.68	

a. Abbreviations: VS--variance source; df--degrees of freedom: MS--mean square; F--Fisher statistic

b. Symbols: **--$P \geq 0.05$; NS--not significant

TABLE 12. BLOOD CELL MORPHOLOGY STATISTICS OF C. auratus L. AT TWO HOLDING DENSITIES.

Characteristic	Group 1 (Mean ± SE)	Group 2 (Mean ± SE)	95% Least Significant Difference
Length, μ	13.66 ± 0.009	13.62 ± 0.009	0.02
Width, μ	8.52 ± 0.008	8.46 ± 0.008	0.02
Roundness	0.62 ± 0.001	0.62 ± 0.001	NS
Area, μ^2	92.25 ± 0.12	91.26 ± 0.12	0.24

TABLE 13. CALCULATED VALUES FOR AREA AND ROUNDNESS OF RED BLOOD CELLS.

Fish	Area (μ^2)	Roundness	Source
Goldfish	92.25	0.62	Present study, group 1
Goldfish	91.26	0.62	Present study, group 2
Goldfish	92.61	0.66	Watson et al. 1963
Goldfish	83.51	0.61	Smith et al. 1952
Catfish	65.51	0.69	Haws and Goodnight 1962
Brown bullhead	102.70	0.68	Haws and Goodnight 1962

TABLE 14. EFFECTS OF LOADING DENSITY ON CELL MORPHOLOGY OF CHANNEL CATFISH.

Loading Density	Length (μ)	Width (μ)	Roundness	Area (μ^2)
0.18 kg/m^3	10.17 ± 0.04	7.79 ± 0.03	0.783 ± 0.004	62.04 ± 0.33
224 kg/m^3	9.38 ± 0.04	7.30 ± 0.03	0.786 ± 0.004	53.94 ± 0.33
337 kg/m^3	8.70 ± 0.04	7.08 ± 0.03	0.821 ± 0.004	48.52 ± 0.33
433 kg/m^3	8.68 ± 0.04	7.15 ± 0.03	0.830 ± 0.004	48.92 ± 0.33
Between Groups	**	**	**	**
95% L.S.D.	0.11	0.09	0.012	0.91
Among Fish/group	**	**	**	**

**, F>0.0001. Means and standard errors are indicated.

TABLE 15. COMPARISON OF CELL MORPHOLOGY FOR GROUP 1 (50 CELL MEASUREMENTS PER FISH AND GROUP 2 (250 CELL MEASUREMENTS PER FISH).

	Length (μ)	Width (μ)	Roundness	Area (μ^2)
Group 1	10.12 ± 0.04	7.89 ± 0.03	0.792 ± 0.004	62.70 ± 0.35
Group 2	9.77 ± 0.02	7.61 ± 0.01	0.787 ± 0.002	58.49 ± 0.14
C.V. (%)				
1	13.16	12.06	15.64	17.69
2	11.74	9.95	13.56	16.39
t	**	**	NS	**

**, t >0.0001; NS, not significant. Means and standard errors are indicated.

TABLE 16. COMPOSITION OF FISH CULTURES

Group	Type of Culture	No. of Bluegill Adult	No. of Bluegill YOY*	No. of Walleye 3-36 cm	No. of Golden Shiners* 3-8 cm
1	Monoculture	150	0	0	0
2	Biculture	150	9,000	25	0
3	Biculture	150	4,500	50	0
4	Biculture	150	4,500	100	0
5	Triculture	150	4,500	50	10,000

*Estimates at termination of study
YOY = young-of-year

TABLE 17. EFFECTS OF CULTURE COMPOSITION ON BLUEGILL HEMATOLOGY

Group	1	2	3	4	5
Hematocrit (%)	32.63	39.93	42.22	33.77	41.89
Hemoglobin (g/100 ml)	5.93	6.94	6.90	5.55	6.24
Plasma osmolality (mosm/kg)*	296.57	303.79	272.23	300.07	276.93
Glucose (g/100 ml)*	81.86	66.43	63.14	101.86	101.86
Albumin (g/100 ml)*	0.54	0.28	0.60	1.42	1.55
Protein (g/100 ml)*	4.81	5.54	4.64	4.99	4.58
Alb/P (%)*	9.8	4.7	21.3	26.4	32.9
rbc x 10^6 (cells/mm^3)	1.90	2.59	2.59	2.32	2.46
Mean cell volume (μ^3) (MEV)	177.59	164.47	184.24	152.06	186.90
Mean cell hemoglobin (pg) (MEH)	32.95	27.69	28.43	24.69	26.94
Mean cell hemoglobin concentration (%) (MEHc)	19.00	18.50	16.35	16.51	15.44
Cell length (μ)	11.26	11.55	11.99	10.98	11.92
Cell width (μ)	7.15	7.00	7.40	6.92	7.87
Cell roundness	0.641	0.610	0.624	0.637	0.666
Cell area (μ^2)	63.21	63.49	69.82	59.72	73.69
Cell height (μ)	4.22	3.96	4.00	3.87	3.79
Thrombocytes (%)	86.17	80.47	79.51	83.40	86.70
Lymphocytes (%)	10.67	7.70	10.19	9.80	9.73
Neutrophils (%)	2.83	11.57	9.77	6.53	3.30
Eosinophils (%)	0.167	0.033	0.233	0.033	0.133
Basophils (%)	0	0	0	0	0.033
Hemoblasts (%)	0.16	0.23	0.37	0.23	0.10
Fish length (cm)	20.75	16.01	15.99	16.67	16.23

*Not included in MANOVA analysis

TABLE 18. MANOVA TEST CRITERIA FOR THE HYPOTHESIS OF NO OVERALL EFFECT OF GROUP. EXCLUDES DATA FOR PLASMAOSMOLALITY, GLUCOSE, ALBUMIN, PROTEIN AND ALB/P.

	1 vs 2*	5 groups	2 vs 3 vs 4*	1 vs 5
Test criteria				
H = type IV SS & CP matrix				
E = error SS & CP matrix				
P = dependent variables	16	17	16	17
Q = hypothesis df	1	4	2	1
NE = df of E	28	70	42	28
S = min (P, Q)	1	4	2	1
M = 0.5 (ABS(P-Q)-1)	7.0	6.0	6.5	7.5
N = 0.5 (NE-P-1)	5.5	26.0	12.5	5.0
Wilk's criterion	0.2644	0.1057	0.1857	0.1552
F	2.26	2.43	2.23	3.84
Probability > F	0.0722	0.0001	0.0046	0.0111

*also excludes data for basophil distribution

TABLE 19. RESULTS OF UNIVARIATE ANALYSIS OF VARIANCE

	1 vs 2	5 groups	2 vs 3 vs 4	1 vs 5
Hemoglobin	*	**	**	
Hematocrit		**	*	**
Number of rbc	**	*		*
MEV				
MEH		*		
MEHc				*
Cell height				
Cell length		**	**	**
Cell width		**	**	**
Cell roundness		*		
Cell area		**	**	**
Thrombocytes	*	*		
Lymphocytes	*			
Neutrophils	**	**		
Eosinophils				
Basophils	-		-	
Hemoblasts				

* $P \geq 0.05$
** $P \geq 0.01$
- not included in analysis

TABLE 20. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR CULTURE STRESS.

Variable	Percentage of the Total Variation	Cumulative Percentage of the Total Variation
Cell area	14.68	14.68
Cell width	14.58	29.26
Neutrophils	9.53	38.79
Cell length	9.37	48.16
Hematocrit	7.80	55.96
Hemoglobin	7.32	63.28
Cell roundness	5.72	69.00
Thrombocytes	5.66	74.66
MEH	4.97	79.63
RBC	4.90	84.53
Hemoblasts	3.13	87.66
Lymphocytes	2.95	90.61
MEHc	2.89	93.50
Eosinophils	2.81	96.31
Basophils	1.92	98.23
MEV	1.42	99.65
Cell height	0.35	100.00

TABLE 21. RESULTS OF PARTIAL CORRELATIONS COR CULTURE STRESS

	HB	HC	RBC	MEV	MEH	MEHc	CH	CL	CW	CR	CA	THR	LYM	NEU	EOS	BAS	HEM
HB			*	-*		*	-*										
HC				*		-*											
RBC				-*	-*		-*										
MEV					*	-*	*										
MEH							*										
MEHc							-*										
CH																	
CL																	
CW										-*	*						
CR										*	*						
CA																	
THR																	
LYM														-*			
NEU																	
EOS																	
BAS																	
HEM																	

*P = 0.0001

TABLE 22. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR THE CONTROL GROUPS (GROUPS 1 AND 2)

Variable	Percentage of the Total Variation	Cumulative Percentage of the Total Variation
Neutrophils	18.24	18.24
RBC	17.21	35.45
Lymphocytes	10.46	45.91
Hemoglobin	9.83	55.74
Thrombocytes	8.03	63.77
Hematocrit	7.62	71.39
Cell roundness	7.42	78.81
Eosinophils	6.59	85.40
MEH	5.90	91.30
Cell length	4.16	95.46
Cell width	2.21	97.67
Hemoblasts	1.00	98.67
MEV	0.75	99.42
Cell height	0.45	99.87
MEHc	0.10	99.97
Cell area	0.03	100.00

TABLE 23. RESULTS OF PARTIAL CORRELATIONS FOR THE CONTROL GROUPS (GROUPS 1 AND 2)

	HB	HC	RBC	MEV	MEH	MEHc	CH	CL	CW	CR	CA	THR	LYM	NEU	EQS	HEM
HB																
HC						-*										
RBC				-*	-*		-*									
MEV					*		*									
MEH																
MEHc							-*									
CH																
CL											*					
CW										*	*					
CR																
CA																
THR														-*		
LYM																
NEU																
EOS																
HEM																

*P = 0.0001

TABLE 24. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR DENSITY STRESS OF SECOND SPECIES

Variable	Percentage of the Total Variation	Cumulative Percentage of the Total Variation
Cell area	16.32	16.32
Cell length	15.13	31.45
Hemaglobin	12.24	43.69
Cell width	10.86	54.55
Hematocrit	9.13	63.68
Eosinophils	5.68	69.36
Lymphocytes	5.18	74.54
Hemoblasts	4.57	79.11
Neutrophils	4.45	83.56
MEH	4.17	87.73
Cell roundness	3.63	91.36
Thrombocytes	2.30	93.66
MEV	2.24	95.90
MEHc	2.06	97.96
RBC	1.98	99.94
Cell height	0.06	100.00

TABLE 25. RESULTS OF PARTIAL CORRELATION ANALYSIS FOR DENSITY STRESS

	HB	HC	RBC	MEV	MEH	MEHc	CH	CL	CW	CR	CA	THR	LYM	NEU	EOS	HEM
HB			*	-*		*	-*									
HC						-*										
RBC				-*	-*		-*									
MEV					*	-*	*									
MEH							*									
EHc							-*									
CH																
CL										-*	*					
CW										*	*					
CR																
CA																
THR														-*		
LYM																
NEU																
EOS																
HEM																

*P = 0.0001

TABLE 26. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES FOR A COMPLEX CULTURE CONSISTING OF THREE FISH SPECIES

Variable	Percentage of the Total Variation	Cumulative Percentage of the Total Variation
Cell area	22.40	22.40
Cell width	21.58	43.98
Hematocrit	13.35	57.33
Cell length	10.68	68.01
RBC	8.29	76.30
MEHc	7.89	84.19
MEH	5.66	89.85
Cell roundness	3.30	93.15
Basophils	1.62	94.77
Hemoglobin	1.59	96.36
Cell height	1.26	97.62
Lymphocytes	0.87	98.49
Hemoblasts	0.80	99.29
Neutrophils	0.29	99.58
MEV	0.24	99.82
Thrombocytes	0.18	100.00
Eosinophils	0	100.00

TABLE 27. RESULTS OF PARTIAL CORRELATION ANALYSIS FOR TRICULTURE

	HB	HC	RBC	MEV	MEH	MEHc	CH	CL	CW	CR	CA	THR	LYN	NEU	EOS	BAS	HEM
HB																	
HC						-*											
RBC				-*	-*		-*										
MEV					*		*										
MEH							*										
MEHc																	
CH																	
CL										-*	*						
CW										*	*						
CR																	
CA																	
THR													-*	-*			
LYM																	
NEU																	
EOS																	
BAS																	
HEM																	

*P = 0.0001

TABLE 28. SUMMARY OF FISH SIZE AND BLOOD CHEMISTRY OF BLUEGILL.

Treatment		Length (cm)	Hemoglobin (g/100 ml)	Hematocrit (%)	ESR (mm/hr)	Protein (g/100 ml)
Group:	1 (Aug)	13.84 ± 0.14a,b	5.97 ± 0.18a	47.61 ± 1.10a	1.11 ± 0.13	6.57 ± 0.58b
	2 (Nov)	13.54 ± 0.14a	6.43 ± 0.18a,b	46.89 ± 1.10a	1.36 ± 0.13	6.16 ± 0.58b
	3 (Feb)	13.72 ± 0.14a	6.63 ± 0.18b	51.29 ± 1.10b	1.07 ± 0.13	4.30 ± 0.58a
	4 (Jun)	14.17 ± 0.14b	6.62 ± 0.18b	46.54 ± 1.10a	1.08 ± 0.13	5.76 ± 0.58a,b
Effect of Group (G)		*	*	**	NS	*
Sex:	Male (M)	14.08 ± 0.10	6.12 ± 0.12	47.59 ± 0.77	1.13 ± 0.09	6.08 ± 0.41
	Female (F)	13.56 ± 0.10	6.72 ± 0.12	48.58 ± 0.77	1.19 ± 0.09	5.32 ± 0.41
Effect of Sex (S)		**	*	NS	NS	**
Interaction:	G_1M	14.34 ± 0.20	5.91 ± 0.25	49.66 ± 1.55	0.83 ± 0.18	9.11 ± 0.82
	G_2M	13.83 ± 0.20	5.84 ± 0.25	43.74 ± 1.55	1.64 ± 0.18	8.61 ± 0.82
	G_3M	13.69 ± 0.20	6.21 ± 0.25	51.50 ± 1.55	1.01 ± 0.18	5.44 ± 0.82
	G_4M	14.44 ± 0.20	6.52 ± 0.25	45.44 ± 1.55	1.02 ± 0.18	7.24 ± 0.82
	G_1F	13.34 ± 0.20	6.03 ± 0.25	45.56 ± 1.55	1.40 ± 0.18	7.32 ± 0.82
	G_2F	13.26 ± 0.20	7.02 ± 0.25	50.04 ± 1.55	1.08 ± 0.18	6.79 ± 0.82
	G_3F	13.74 ± 0.20	7.04 ± 0.25	51.07 ± 1.55	1.12 ± 0.18	5.32 ± 0.82
	G_4F	13.90 ± 0.20	6.71 ± 0.25	47.65 ± 1.55	1.15 ± 0.18	7.16 ± 0.82
Effect of GS		NS	NS	**	*	NS

*$P \geq 0.05$

**$P \geq 0.001$

NS = not significant. Similar letters between means indicate no significant difference as determined by the 95 percent Least Significant Difference test.

TABLE 29. SUMMARY OF HEMATOLOGICAL INDICES OF BLUEGILL.

Treatment		rbc x 10^6 (cells/mm^3)	MEV (cμ)	MEH (μμg)	MEHc (%)
Group:	1 (Aug)	2.83 ± 0.09a	177.33 ± 7.06a	22.40 ± 1.14a	13.03 ± 0.44
	2 (Nov)	2.98 ± 0.09a	159.92 ± 7.06a	22.17 ± 1.14a	13.91 ± 0.44
	3 (Feb)	2.51 ± 0.09b	211.04 ± 7.06b	27.14 ± 1.14b	12.99 ± 0.44
	4 (Jun)	2.48 ± 0.09b	197.64 ± 7.06b	28.46 ± 1.14b	14.40 ± 0.44
Effect of Group (G)		**	**	**	NS
Sex:	Male (M)	2.56 ± 0.06	194.22 ± 4.99	25.19 ± 0.80	13.11 ± 0.31
	Female (F)	2.84 ± 0.06	178.74 ± 4.99	24.89 ± 0.80	14.06 ± 0.31
Effect of Sex (S)		*	*	NS	*
Interaction:	G_1M	2.76 ± 0.13	191.13 ± 9.98	22.94 ± 1.14	12.26 % 0.63
	G_2M	2.71 ± 0.13	165.11 ± 9.98	22.20 ± 1.14	13.48 ± 0.63
	G_3M	2.45 ± 0.13	216.48 ± 9.98	26.06 ± 1.14	12.09 ± 0.63
	G_4M	2.32 ± 0.13	204.48 ± 9.98	29.57 ± 1.14	14.59 ± 0.63
	G_1F	2.90 ± 0.13	163.53 ± 9.98	21.86 ± 1.14	13.81 ± 0.63
	G_2F	3.26 ± 0.13	154.74 ± 9.98	22.15 ± 1.14	14.34 % 0.63
	G_3F	2.56 ± 0.13	205.60 ± 9.98	28.22 ± 1.14	13.91 ± 0.63
	G_4F	2.65 ± 0.13	191.10 ± 9.98	27.35 ± 1.14	14.20 ± 0.63
Effect of GS		NS	NS	NS	NS

*$P \geq 0.05$
**$P \geq 0.001$
NS = not significant. Similar letters between means indicate no significant difference as determined by the 95 percent Least Significant Difference test.

TABLE 30. SUMMARY OF LEUCOCYTE-THROMBOCYTE DISTRIBUTION (%) OF BLUEGILL BLOOD.

Treatment		Cell Type					
		Thrombocytes	Lymphocytes	Neutrophils	Eosinophils	Basophils	Hemoblasts
Group:	1 (Aug)	90.12 ± 0.93a	7.79 ± 0.50a	4.22 ± 0.83a	0.50 ± 0.05	0.0000 ± 0.0063	0.21 ± 0.06
	2 (Nov)	89.12 ± 0.93a	8.41 ± 0.50a	4.02 ± 0.83a	0.11 ± 0.05	0.0000 ± 0.0063	0.12 ± 0.06
	3 (Feb)	80.72 ± 0.93b	10.09 ± 0.50b	8.79 ± 0.83b	0.24 ± 0.05	0.0000 ± 0.0063	0.16 ± 0.06
	4 (Jun)	81.34 ± 0.93b	12.67 % 0.50c	5.62 % 0.83a	0.11 ± 0.05	0.1200 ± 0.0063	0.24 ± 0.06
Effect of Group (G)		**	**	**	NS	NS	NS
Sex:	Male (M)	84.84 ± 0.66	10.11 ± 0.36	5.71 ± 0.59	0.08 ± 0.03	0.0000 ± 0.0045	0.16 ± 0.04
	Female (F)	85.82 ± 0.66	9.37 ± 0.36	5.62 ± 0.59	0.17 ± 0.03	0.0062 ± 0.0045	0.21 ± 0.04
Effect of Sex (S)		NS	NS	NS	*	NS	NS
Interaction:	G_1M	90.30 ± 1.32	7.87 ± 0.71	1.60 ± 1.18	0.05 ± 0.07	0.0000 ± 0.0089	0.17 ± 0.08
	G_2M	89.60 ± 1.32	8.70 ± 0.71	5.25 ± 1.18	0.02 ± 0.07	0.0000 ± 0.0089	0.02 ± 0.08
	G_3M	78.72 ± 1.32	10.25 ± 0.71	10.72 ± 1.18	0.12 ± 0.07	0.0000 ± 0.0089	0.17 ± 0.08
	G_4M	80.72 ± 1.32	13.62 ± 0.71	2.68 ± 1.18	0.12 ± 0.07	0.0000 ± 0.0089	0.27 ± 0.08
	G_1F	89.95 ± 1.32	7.70 ± 0.71	6.85 ± 1.18	0.05 ± 0.07	0.0000 ± 0.0089	0.25 ± 0.08
	G_2F	88.65 ± 1.32	8.12 ± 0.71	4.07 ± 1.18	0.20 ± 0.07	0.0000 ± 0.0089	0.22 ± 0.08
	G_3F	82.75 ± 1.32	9.92 ± 0.71	6.85 ± 1.18	0.35 ± 0.07	0.0000 ± 0.0089	0.15 ± 0.08
	G_4F	81.95 ± 1.32	11.72 ± 0.71	6.00 ± 1.18	0.10 ± 0.07	0.0250 ± 0.0089	0.20 ± 0.08
Effect of GS		NS	NS	**	NS	NS	NS

*$P \geq 0.05$

**$P \geq 0.001$

NS = not significant. Similar letters between means indicate no significant difference as determined by the 95 percent Least Significant Difference test.

TABLE 31. SUMMARY OF RED BLOOD CELL MORPHOLOGY OF BLUEGILL.

Treatment		Cell Length (µm)	Cell Width (µm)	Cell Height (µm)	Cell Roundness	Cell Area (μm^2)
Group 1 (Aug)		10.91 ± 0.12a	7.63 ± 0.08b	4.13 ± 0.17b	0.707 ± 0.008b	65.53 ± 1.26a
2 (Nov)		11.86 ± 0.12b	7.34 ± 0.08a	3.53 ± 0.17a	0.625 ± 0.008a	68.31 ± 1.26a,b
3 (Feb)		11.98 ± 0.12b	7.52 ± 0.08a,b	4.54 ± 0.17b	0.636 ± 0.008a	70.63 ± 1.26b
4 (Jun)		12.44 ± 0.12c	8.95 ± 0.08c	3.42 ± 0.17a	0.726 ± 0.008c	87.54 ± 1.26c
Effect of Group (G)		**	**	**	**	**
Sex Male (M)		11.84 ± 0.08	7.80 ± 0.06	4.06 ± 0.12	0.666 ± 0.006	72.55 ± 0.89
Female (F)		11.76 ± 0.08	7.92 ± 0.06	3.75 ± 0.12	0.681 ± 0.006	73.46 ± 0.89
Effect of Sex (S)		NS	NS	NS	NS	NS
Interaction:	G_1M	11.06 ± 0.17	7.74 ± 0.12	4.33 ± 0.24	0.707 ± 0.011	67.40 ± 1.78
	G_2M	12.01 ± 0.17	7.38 ± 0.12	3.58 ± 0.24	0.621 ± 0.011	69.52 ± 1.78
	G_3M	11.99 ± 0.17	7.37 ± 0.12	4.73 ± 0.24	0.622 ± 0.011	69.44 ± 1.78
	G_4M	12.28 ± 0.17	8.69 ± 0.12	3.61 ± 0.24	0.715 ± 0.011	83.84 ± 1.78
	G_1F	10.76 ± 0.17	7.52 ± 0.12	3.93 ± 0.24	0.707 ± 0.011	63.66 ± 1.78
	G_2F	11.73 ± 0.17	7.29 ± 0.12	3.48 ± 0.24	0.629 ± 0.011	67.10 ± 1.78
	G_3F	11.96 ± 0.17	7.66 ± 0.12	4.36 ± 0.24	0.650 ± 0.011	71.83 ± 1.78
	G_4F	12.60 ± 0.17	9.21 ± 0.12	3.23 ± 0.24	0.737 ± 0.011	91.25 ± 1.78
Effect of GS		NS	NS	NS	NS	NS

**$F \geq 0.0001$

NS = not significant. Similar letters between means indicate no significant difference as determined by the 95 percent Least Significant Difference test.

TABLE 32. RESULTS OF PARTIAL CORRELATION ANALYSIS.

	HB	HC	ESR	RBC	MEV	MEH	MEHc	CL	CW	CR	CA	CH	THR	LYM	NEU	EOS	BAS	HEM
HB						*	*											
HC			-*	*	*		-*					*						
ESR							*											
RBC					-*	-*						-*						
MEV						*						*						
MEH							*					*						
MEHc																		
CL									*	-*	*	-*						
CW										*	*	-*						
CR																		
CA												*						
CH																		
THR														-*	-*			
LYM																		
NEU																		
EOS																		
BAS																		
HEM																		

*P $\geq$ 0.001

TABLE 33. MANOVA TEST CRITERIA FOR THE HYPOTHESIS OF NO OVERALL EFFECT FOR SEX, SEASON, AND SEX-SEASON INTERACTION.[1]

Test Criteria	Sex	Season	Sex-Season
H = type IV SS and CP			
E = error SS and CP matrix			
P = dependent variables	18	18	18
Q = rank of H	1	3	3
NE = DF of E	152	152	152
S = Min (P,Q)	1	3	3
M = 0.5 !ABS (P-Q) -11	8.0	7.0	7.0
N = 0.5 (NE -P -1)	66.5	66.5	66.5
Wilk's criterion	0.7875	0.0951	0.3627
F	(18,135) 2.02	(54,403) 8.98	(54,403) 0.033
Probability >F	0.0123	0.0001	0.0001

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[1] Excludes data for fish length and plasma protein.

TABLE 34. PERCENTAGE OF THE TOTAL VARIATION OF THE HEMATOLOGICAL VARIABLES.

Variable	Percentage of the total variation	Cumulative percentage of the total variation
Cell width	14.25	14.25
Cell area	13.43	27.68
Cell roundness	10.91	38.59
Cell length	9.00	47.59
Thrombocytes	8.86	56.45
Lymphocytes	6.72	63.17
Neutrophils	4.79	67.96
MEV	4.55	72.51
RBC	4.48	76.99
Cell height	4.31	81.30
MEH	3.48	84.78
Hemoglobin	3.44	88.22
Hematocrit	3.31	91.53
Eosinophils	2.36	93.89
MEHc	2.19	96.08
ESR	1.90	97.98
Basophils	1.04	99.02
Hemoblasts	0.98	100.00

TABLE 35. SOME BLOOD PARAMETERS OF BLUEGILL.

Variable	No. of fish	Measurement	Reference
HC	12	53.1%	Burton 1979
HB	27	7.6-7.9 g/100 ml	Smith et al. 1952
RBC	27	2.6×10^6 cell/mm^3	Smith et al. 1952
MEH	27	29.4 mmg	Smith et al. 1952
CL	1	10.9 mm	Smith et al. 1952
CW	1	6.4 mm	Smith et al. 1952

SECTION 6

A SELECTED AND ANNOTATED BIBLIOGRAPHY OF BASELINE AND TEMPERATURE STUDIES ON FISH BLOOD

Ahokas, R. A. and G. Sorg. 1977. The effect of salinity and temperature on intracellular osmoregulation and muscle free amino acids in *Fundulus diaphanus*. *Comp. Biochem. Physiol.*, 56A:101-105.

Intracellular osmoregulation and tissue free amino acids were examined in *Fundulus diaphanus* subjected to different combinations of temperature (0.5° and 15°C) and salinity (0% and 30% seawater). Although fish held at 15°C tolerated salinity well, 50% of the fish held at 0.5°C in 30% seawater died. Plasma osmolarity increased with an increase in ambient salinity at both temperatures. Salinity increases at 0.5°C resulted in decreased body water content, and increased muscle sodium, chloride and potassium levels. Adaptation to seawater at 15°C resulted in elevated body water, tissue electrolytes, and free amino acids. In freshwater adapted fish, decreased temperatures resulted in elevated tissue total inorganic ions, and potassium levels, and reduced free amino acid levels. The changes in amino acids due to temperature were discussed.

Arkin, H., and R. R. Colton. 1963. *Tables for Statisticians*. 2nd ed. Barnes and Noble, Inc., New York. 168 p.

Banks, J. L., L. G. Fowler, and J. W. Elliott. 1971. Effects of rearing temperature on growth, body form, and hematology of fall chinook fingerlings. *Prog. Fish-Cult.* 33:20-26.

The effect of rearing temperature on growth, and hematology was investigated in fingerling chinook reared at 50°, 55°, 60°, and 65°F. Weight gains were greater at 60°F than at 50° and 55°F. Temperature had no apparent effect on body form. Temperature effects on fish hematology were found only in the smallest fish. In these fish, elevated rearing temperature resulted in increased corpuscle counts, hematocrit and hemoglobin values.

Barnhart, R. A. 1969. Effects of certain variables on hematological characteristics of rainbow trout. *Trans. Amer. Fish. Soc.* 3:411-418.

Comparative hematological studies were performed on Shasta and Idaho strains of rainbow trout fed two different commercial diets (Colorado and Shasta). The blood parameters investigated were total blood cell count, hematocrit, hemoglobin, erythrocyte sedimentation rate, bilirubin, alkaline phosphate, glutamic oxaloacetic transaminase, total protein, glucose, cholesterol, creatinine, and uric acid levels.

There were no sex-related differences found in the hematological values. Age-related variations appeared to be random. Significant differences in many of the blood components were associated with diet, strain, and/or diet-strain interactions. In addition, hematological differences were found between "wild" and cultured fish.

Bartlett, G. R. 1976. Phosphate compounds in red cells of reptiles, amphibians and fish. *Comp. Biochem. Physiol.* SSA:211-214.

The concentrrations of 2, 3 diphosphoglycerate (DPG) inositol pentaphosphate (IP5) and ATP were examined in erythrocytes of reptiles, amphibians, and fish.

Bennett, M. F., and C. G. Neville. 1975. Effects of cold shock on the distribution of leucocytes in goldfish, *Carassius auratus*.

Goldfish, which were acclimated to 22°-24°, were exposed to 2°C for 3.5-4.0 minutes and then returned to the acclimation temperatures. Animals were sacrificed and blood was sampled at 1, 2, 4, 6, and 24 hours after cold shock. When the leucocyte differential counts of the control fish were compared to those of the cold shock fish, there appeared to be significant lymphopenia and neutrophilia in experimental fish during the first and second hours after treatment. The counts for the experimental fish and the control fish were not significantly different by four hours after treatment.

Blaxhall, P. C. and K. W. Daisley. 1973. Routine hematological methods for use with fish blood. *J. Fish Biol.* 5:771-781.

Routine methods for examining fish hematology were described. Hematology of healthy 2-year old brown trout was investigated. The means and standard errors for hematocrit, hemoglobin, erythrocyte counts, erythrocyte sedimentation rate, and total and differential leucocyte counts were presented. Descriptions were given of the blood cell types observed in some teleosts.

Block, R. M. 1974. Effects of acute cold shock on the channel catfish. In: Gibbons, J. W. and R. R. Sharitz (eds.), *Thermal Ecology Proceedings of a Symposium Held at Augusta, Georgia, May 3-5, 1973*. U.S. Atomic Energy Commission, Oak Ridge, Tn., pp. 109-118.

Blood chemistry changes were observed in cold shocked channel catfish. Plasma osmolarity and chloride values rose within 10 minutes after fish acclimated to 30°C were abruptly transferred to 10°C. After 24 hours, osmolarity and chloride values decreased rapidly, concomitant with elevated plasma glucose and muscle water levels. When cold shocked fish were returned to 30°C, plasma glucose, osmolarity and chloride values slowly returned to normal. Possible mechanisms of cold death were discussed. The applicability of monitoring blood chemistry to detect stress was discussed.

Bonaventura, C., B. Sullivan, J. Bonaventura, and M. Brunori. 1976. Studies on the root effect of a marine teleost. *J. Biol. Chem.* 251(7):1871-1876.

The Spot, Leiostomus xanthurus, had a single tetrameric hemoglobin, which possessed a Root effect. The alpha- and beta-chains of the Spot hemoglobin had a similar molecular weight and the same COOH-terminal residues as those observed in human hemoglobins. The Root effect may be a molecular adaption to help the fish attain neutral bouyancy, in that hemoglobins sensitive to pH and anions may facilitate oxygen delivery to the swimbladder Reduced ligand affinity of Spot hemoglobin at low pH values may be partially attributed to kinetic changes in the "on" and "off" processes. At low pH levels the ligand combination process was biphasic and wavelength dependent, suggesting that there was differential pH sensitivity of the alpha- and beta-chains. This may have contributed to observed pH dependence of the ligand-binding curve. Changes in the ligand-binding curve with pH levels may also have been associated with protein-dependent transition between low(T) and high(R) affinity conformations.

Brooke, H. E. 1964. A Review of variations found in fish serum proteins. N.Y. Fish and Game J. 11(1):47-57.

Review of the literature concerning causes of variation in serum proteins.

Boyar, H. C. 1962. Blood cell types and differential cell counts in Atlantic herring, Clupea harengus harengus. Copeia 2:463-465.

Blood cell differential counts were made on blood from brit (75 to 100 mm), juvenile (101 to 200 mm) and adult (ripe and spent) Atlantic herring (201 to 290 mm). Mature erythrocytes comprise 97% of the total blood count. Lymphocytes and basophils were the next most common cell types found. Other cell types observed were immature erythrocytes, ghost cells, spindle cells, eosinophils, basophils and neutrophils. No significant differences in the occurrence of cell types could be associated with fish length. Descriptions and sizes of cell types are discussed in the paper.

Burton, C. G. 1979. The effect of fluctuating temperature cycles on osmoregulation in bluegill, Lepomis macrochirus. Masters Thesis, University of Kansas.

The effect of diurnal temperature cycles on osmoregulation was studied in bluegill. Acclimation to temperature cycles resulted in decreased hematocrit levels, with no significant change in plasma osmolarity.

Burton, C. G. and S. A. Murray. 1979. Density effects on goldfish blood. I. Hematology. Comp. Biochem. and Physiol. 62A:555-558.

The effect of loading density (0.8 and 4.19 fish centimeters/liter of water) on the blood chemistry was studied in goldfish. Increased fish loading density resulted in decreased hematocrit and hemoglobin levels and increased erythrocyte sedimentation rates. A significant decrease in hemoglobin values was associated with the higher density of males. Fish size and sex appeared to mask density effects on blood chemistry. There were no significant differences in mean erythrocytic volume, mean erythrocytic hemoglobin concentration, nor plasma osmolarity values between the two density groups.

Cairns, J., Jr. and A. Scheier. 1964. Blood corpuscle count, hemoglobin concentration and oxygen consumption values for the pumpkinseed sunfish, *Lepomis gibbosus* (Linn.). *Notulae Naturae of the Academy of Natural Sciences of Philadelphia*: (366):1-4.

Blood corpuscle counts, hemoglogin and oxygen consumption values were measured in pumpkinseed sunfish, which were held in the laboratory at 20°C for 3-7 days. The mean hemoglobin concentration was 10.15 g/100 ml, and the mean corpuscular count was 2.29 x 10^6 cells/mm^3. After 30 minutes of activity the mean oxygen consumption value was 7.65 mg oxygen.

Cameron, J. N. 1970. The influence of environmental variables on the hematology of Pinfish (*Lagodon rhomboides*) and striped mullet (*Mugil cephalus*). *Comp. Biochem. Physiol.* 33:175-192.

A field population of *Mugil cephalus* had elevated hemoglobin concentrations, hematocrits, and erythrocyte counts, with no change in mean erythrocyte volumes, in response to high environmental temperature. A field population of *Logodon rhomboides* had elevated hemoglobin concentrations, and erythrocyte counts and decreased hematocrit and mean erythrocyte volumes at high temperatures. Pinfish acclimated to 25°C in the laboratory had a similar increase in hemoglobin concentrations, but no change in hematocrits. Acclimation of pinfish to low oxygen, high salinity and exercise resulted in elevated blood concentration.

The elevated hemoglobin concentrations at high temperatures in both species probably increased the oxygen transport by only about 15%. As this was relatively small when compared to the change in total respiratory demand, other changes in the blood may have aided in respiration.

Cattlett, R. H., and D. R. Millich. 1976. Intracellular and extracellular osmoregulation of temperature acclimated goldfish: *Carassius auratus* L. *Comp. Biochem. Physiol*. 55A:261-269.

Intracellular and extracellular osmoregulation was investigated in goldfish acclimated to temperatures ranging from 1°C to 21.5°C. Cold acclimation resulted in low plasma osmolarity due to reduced organic solutes and inorganic ions. Exposure to cold temperatures resulted in decreased plasma sodium concentrations, hematocrits, erythrocyte counts, and mean corpuscular fragility. Plasma chloride concentrations were slightly elevated at 1°C and were relatively stable from 5° to 21.5°C. The erythrocyte response to lower plasma osmolarity values was characterized by osmotic swelling followed by cellular shrinkage, which was apparently caused by an efflux of potassium chloride and water.

Chlebeck, A. and G. L. Phillips. 1969. Hematological study of two buffalofishes _Ictiobus_ _cyprinellus_ and _I_. _bubalus_ (Catostomidae). _J. Fish Res. Bd. Can._ 26(11):2881-2886.

A comparative hematological study was performed on _Ictiobus cyprinellus_ and _I_. _bubalus_. Hemoglobin concentrations, hematocrits, mean corpuscle hemoglobin levels, erythrocyte counts and, leucocyte counts were measured in both species of Catostomid fish. Lymphocytes, neutrophils, "secretory granulocytes," eosinophils and thrombocytes were the leucotyic cell types present. No basophils were observed.

Conroy, D. A. 1972. Studies on the haematology of the Atlantic Salmon (_Salmo_ _salar_ L.). _Symp. Zool. Soc. Lond._ (30):101-127.

Hematology as studied in wild and hatchery-reared _Salmo_ _salar_ at different ages and stages of life. Hematological values were obtained for freshwater and saltwater salmon. The erythrocyte counts remained fairly stable during all stages of the life cycle. The blood of seawater salmon was more concentrated than blood of freshwater salmon. Prespawning migrants had higher hemoglobin levels than kelts and immature salmon. No apparent hematological differences were found between salmon parr and smolts, and similar hematological values were found for kelts and immature salmon in freshwater. Peripheral blood of Atlantic salmon contained erythrocytes, thrombocytes, lymphocytes, neutrophils, eosinophils and macrophages. Erythrocyte size and shape at different stages of the life cycle were also discussed.

Courtois, L. A. 1975a. Hematological assessment and toxicity of antimycin A to golden shiners, _Notemigonus_ _cyrsoleucas_. _Prog. Fish-Cult_. 37(4):202-204.

The hematology of "wild" and laboratory-acclimated golden shiners were compared. In addition, a bioassay was performed to determine the effective level of antimycin A necessary to control this species during the autumn. No significant differences in hematocrit, hemoglobin, and serum protein levels were found between "wild" and laboratory-acclimated fish. Toxicity studies revealed that 1.0 µg/ℓ of antimycin A produced 100% mortality in golden shiners. Lower concentrations were not 100% effective.

Courois, L. A. 1975b. Blood and serum analyses of adult striped bass, _Morone_ _saxatilis_, captured in the Sacramento River. _Calif. Fish and Game_ 61(4):245-246.

Analyses were performed on the blood of migrating striped bass captured in the Sacramento River. Hematocrit, hemoglobin, serum sodium, potassium, calcium, cholesterol, inorganic phosphate, blood urea nitrogen, glucose, uric acid, total protein, and alkaline phosphate values were given.

Courtois, L. A. 1976. Hematology of juvenile striped bass, *Morone saxatilis* (Walbaum), acclimated to different environmental conditions. *Comp. Biochem. Physiol.* 54A:221-223.

> Acclimation of juvenile striped bass to different temperature and salinity conditions resulted in changes in hematology. Fish acclimated to seawater had lower hematocrit and hemoglobin levels and higher serum sodium levels than freshwater-acclimated fish. There was also a reduction in serum potassium in seawater-acclimated fish, which was significant in warmwater-acclimated fish. Elevated temperature resulted in reverse effects in freshwater- and seawater-acclimated striped bass. Plasma protein was increased and serum sodium decreased in warm seawater-acclimated fish, whereas plasma protein was decreased and serum sodium increased in warm freshwater-acclimated fish.

Das, B. C. 1964/65. Age-related trends in the blood chemistry and hematology of the Indian carp *Catla catla*. *Gerontologia* 10:47-64.

> The effect of length-at-age on blood chemistry and hematology was studied in pond-cultured Indian carp (*Catla catla*). The fish were sampled between 210 and 529 days of age. They were placed into seven length-at-age groups. Amylase activity, chlorides, creatinine, glucose, total protein, and hemoglobin, levels, and erythrocyte counts, and cell type A counts appeared to increase with length-at-age. There was a decrease with length-at-age found for calcium, and acid and alkaline phosphatase levels. Total cholesterol, free cholesterol, cholesterol ester, nonprotein nitrogen, urea, and uric acid levels appeared to increase initially with length-at-age and then decrease. No relationship was found between cell type B or C and length-at-age.

Davis, K. B. and B. A. Simco. 1976. Diurnal measurements of plasma chloride in catfish. *Copeia* (3):608-609.

> Diurnal plasma chloride concentrations were studied in *Ictalurus punctatus* during winter, spring and autumn. Fish were held in raceways, outdoor tanks, or open ponds. There was no diurnal variation found in plasma chloride concentrations during any season nor under any of the environmental conditions examined. Although mean diurnal plasma chloride concentrations were significantly lower in May than in any other month, it was not known whether this was the result of environmental conditions or seasonal influences. Density appeared to have no significant effect on plasma chloride concentration.

Davis, K. B. and B. A. Simco. 1976. Salinity effects on plasma electrolytes of channel catfish *Ictalurus punctatus*. *J. Fish. Res. Bd. Can.* 33(4, part 1):741-746.

The effects of salinity and temperature on hematology was investigated in channel catfish. When channel catfish were exposed to 10 and 12 g/ℓ of sodium chloride for 5 days, the plasma sodium and chloride concentrations were elevated. After 5 days exposure to 4 and 8 g/ℓ of sodium chloride, plasma electrolyte levels were higher than those of catfish held in aged tapwater, but were not significantly different from values found in freshwater catfish. These differences may have been associated with handling of the catfish. Although the tests were performed during several months, no seasonal differences in plasma electrolytic values were found.

When catfish were exposed to 10 g/ℓ sodium chloride at 27°C during July, the plasma electrolyte levels increased during the first 48 hours, and then remained relatively constant. The hematocrit levels decreased throughout the experimental period. Plasma solids and protein levels increased for 1 day, and then returned to normal.

Exposure of channel catfish to 10 g/ℓ sodium chloride and 9°C during March, resulted in a gradual increase in plasma electrolyte values throughout the experimental period. During the same period, hematocrit levels increased initially, decreased for 8 days and then began fluctuating.

Cortisol injections had no significant effect on the elevated plasma chloride, osmolarity and protein levels of catfish transferred from freshwater to 12 g/ℓ of sodium chloride.

Dean, J. M. and L. J. Goodnight. 1964. A comparative study of carbohydrate metabolism in fish as affected by temperature and exercise. Physiol. Zool. 37:280-299.

The effects of temperature (5° and 20°C) and exercise on the tissue and blood was studied in four species of fish (black bullhead, white crappie, bluegill, and largemouth bass). The physiological parameters examined include blood glucose, blood lactic acid, muscle glycogen and liver glycogen.

Exercise resulted in elevated blood lactic acid and blood glucose levels in all species at both 20° and 5°C. In addition, muscle glycogen levels decreased with exercise in all species at both temperatures, except for 5°C acclimated bluegill. The liver glycogen content of black bullhead and largemouth bass at both temperatures and bluegill at 20°C decreased with exercise, while that of white crappie at both temperatures and bluegill at 5°C increased with exercise.

Exposure to cold temperature resulted in elevated serum glucose levels in crappie and bullhead and decreased glucose levels in bluegill. There was no significant change in blood glucose levels of bass associated with temperature.

Denton, J. E. and M.K. Yousef. 1975. Seasonal changes in hematology of rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* 51A:151-153.

When rainbow trout were held at 11°-13°C over an eleven month period, seasonal variations were found in hemoglobin, hematocrit, serum protein, mean erythrocytic volume, mean erythrocyte hemoglobin, and mean erythrocyte hemoglobin concentration. Probable causes of these seasonal variations include diet, metabolic adaptions and activity.

Denyes, H. A. and J. M. Joseph. 1956. Relationships between temperature and blood oxygen in the largemouth bass. *Wild. Man.* 20:56-64.

Temperature effects on the blood of largemouth bass were examined. Bass acclimated to 18°-24°C were exposed to 5°-10°C, 30°, 32°-33°C or 35°C for periods up to 2 hours. The temperature effects on hemoglobin, erythrocyte count, arterial blood oxygen content, oxygen capacity and lactic acid levels were measured. There was no significant correlation between the oxygen content of the water and that of the blood. Arterial blood oxygen content was related to water temperature. Increased opercular pulse rate, hemoglobin content, and lactic acid levels were found in fish exposed to high temperatures and quiesence. There appeared to be a general increase in hemoglobin with increased erythrocyte number. At various temperatures the combining power of hemoglobin with oxygen appeared to be improved by elevated lactic acid levels.

DeVries, A. L., and D. E. Wohlschlag. 1969. Freezing resistance in some Antarctic fishes. *Science* 161:1073-1075.

Serum samples were analyzed in three species of Antarctic marine fish. Freezing point measurements indicated that the fish may not freeze at subzero temperatures because their blood is osomotic to seawater. Deepwater fish had higher serum freezing points than shallow water fish of the same species. Only half of the freezing point depression of the serum was accounted for by sodium chloride, urea and free amino acid levels. Another 30% of the freezing point depression of the serum was attributed to a glycoprotein.

DeWilde, M. A. and A. H. Houston. 1967. Hematological aspects of the thermoacclimatory process in the rainbow trout, *Salmo gairdneri*. *J. Fish Res. Bd. Can.* 24(11):2267-2281.

Thermoacclimatory changes in the hematology of rainbow trout, *Salmo gairdneri*, was investigated in summer and fall-winter fish. Erythrocyte abundance, hematocrit, hemoglobin, mean erythrocytic volume, and mean erythrocytic hemoglobin content were measured at temperatures ranging from 3° to 21°C. Acclimation to cold temperatures resulted in low mean erythrocytic hemoglobin content, erythrocyte counts, hematocrits, and hemoglobin levels and high mean erythrocytic volume. Animals held at intermediate temperatures generally had a greater oxygen-carrying capacity than cold-acclimated trout. Trout acclimated to 21°C had higher hemoglobin, hematocrits, erythrocyte counts, and mean erythrocytic hemoglobin content than fish held at lower temperatures.

There were significant seasonal differences in hemoglobin levels between summer and fall-winter trout.

Dickinson, L. C. and J.C.W. Chien. 1977. Electron paramagnetic resonance study of carp methemoglobin. J. Biol. Chem. 252 (4):1327-1330.

The electron paramagnetic resonance of carp methemoglobin was studied. When pH was decreased, there was a monotonic increase of g anistrophy of the electron paramagnetic resonance spectra of carp azidomethemoglobin, whereas there was no significant variation in the spectra of human azidomethemoglobin. Human and carp azidomethemoglobin had identical g values at pH levels of 8 to 9. Crystal field analysis revealed a rhombic distortion value of 2.25 and a tetragonal distortion value of 3.31 for carp azidomethemoglobin. At pH of 4.0, the rhombic distortion value of carp azidomethemoglobin was 1.95 and the tetragonal distortion value was 4.76, while those for human hemoglobin were almost constant. The data suggested that at low pH levels there was an increase in out-of-place movement of the iron atom and stabilization of the T form of carp azidomethemoglobin. At low pH levels, electron paramagnetic resonance spectra showed that approximately 1.5% of the carp azidomethemoglobin was found to be in the high spin state. Changes in pH did not affect the electron magnetic resonance spectra of aquo- and fluoro-derivatives of carp methemoglobin.

Doumas, B. T., W. A. Watson, and H. G. Biggs. 1971. Clin. Chim. Acta 31:87.

Dow, D., and P.V.C. Pinto. 1969. Clin. Chem. 15:1006.

Edelstein, S. J., B. McEwen, and Q. H. Gibson. 1976. Subunit dissociation in fish hemoglobins. J. Biol. Chem. 251(23):7632-7637.

Sedimentation velocity and flash photolysis measurements were used to study the tetramer-dimer dissociation equilibrium ($K_{4,2}$) of some fish hemoglobins. Detailed studies were done on the hemoglobins from menhaden, carp and blue shark. Sedimentation coefficients of 4.3S in micromolar concentration range were found for CO-hemoglobins of all 3 species. These coefficients were typical of tetrameric hemoglobins. Dissociation was not detected at the lowest concentration studied, suggesting that the $K_{4,2}$ value was equal to 10^{-8} M or below.

Fish photolysis studies detected two kinetic components, which varied in proportions similar to that expected for tetramer-dimer equilibrium. The hemoglobins of the 3 species had calculated $K_{4,2}$ values of 10^{-9} to 10^{-8} M. The liganded forms of the fish hemoglobins appeared to have $K_{4,2}$ values midway between those of liganded forms and unliganded forms of human hemoglogin. Studies on solutions with guanidine hydrochloride supported this conclusion. Variations in the rates and proportions of the fast (dimer) and slow (tetramer) components were suggested from kinetic studies on solutions with guanidine hydrochloride present. Studies on solutions containing urea were also done. However, these were not continued, because at the high urea concentrations needed to encourage dissociation, the hemoglobins were unstable and there was considerable loss of the absorbance spectra in the sedimentation and kinetic studies.

Engel, D. W. and E. M. Davis. 1964. Relationship between activity and blood composition in certain marine teleosts. Copeia (3):586-587.

Hematology was studied in benthic and pelagic marine telestean species. Pelagic species had higher erythrocyte counts, hemoglobin values and hematocrit levels than benthic species. Significant interspecific differences were found for erythrocyte counts within pelagic and benthic species groups. There was no correlation found between activity level of the fish and thrombocyte or leucocyte counts.

Falkner, N. W. and A. H. Houston. 1966. Some hematological responses to sublethal thermal shock in the goldfish, *Carassius auratus* L. J. Fish Res. Bd. Can. 23(8):1109-1120.

There was no significant trend in erythrocyte numbers or hematocrit during a 10-day period after goldfish acclimated to 20°C were abruptly transferred to 30°C. An initial decrease in mean erythrocyte volume following heat shock, was followed by a return to normal values. During the latter portion of the observation period, the mean blood iron and the mean erythrocyte iron content decreased. The blood of goldfish acclimated to 12°, 20°, or 30°C was characterized by three hemoglobin polymorphs, while only two polymorphs were present in fish acclimated to 5°C. The relationships between the hematological observations and respiratory acclimation to elevated temperatures were discussed.

Field, J. B., C. A. Elvehjem, and C. Juday. 1943. A study of the blood constituents of carp and trout. J. of Biol. Chem. 148:261-269.

A comparative study was done on the hematology of carp and trout. Blood samples from carp had significantly higher pH, albumin-globulin ratio, hemoglobin, carotene and vitamin C content and significantly lower uric acid, creatine and vitamin A content than trout.

Fish blood had lower erythrocyte count, and higher mean erythrocyte volume, mean erythrocyte hemoglobin and total blood lipid and cholesterol levels than mammalian blood. In addition, the nonprotein nitrogen present in fish blood was mainly composed of amino acids.

Fletcher, G. L. 1975. The effects of capture, "stress," and storage of whole blood on the red blood cells, plasma proteins, glucose and electrolytes of the winter flounder (*Pseudopleuronectes americanus*). *Can. J. Zool.* 53:197-206.

Many hematological differences were found between blood of laboratory-maintained winter flounder and flounder captured in the field. Field flounder had significantly higher values for hemoglobin, hematocrit, mean erythrocyte volume, plasma osmolarity, plasma sodium, and plasma chloride and significantly lower values for mean erythrocytic hemoglobin content and plasma potassium. There were no significant differences in erythrocyte number, mean erythrocytic hemoglobin, and plasma protein values.

Laboratory experiments on the effects of "stress" revealed that changes in plasma osmolarity, plasma protein, plasma sodium, plasma chlorides, red blood cell numbers, hemoglobin, and mean erythrocytic hemoglobin may have been the result of capture and transportation of field flounder.

Another experiment revealed that the decreased plasma potassium values in field flounder may be the result of storage of whole blood prior to analysis. Alterations in hematocrit, mean erythrocyte volume and mean erythrocytic hemoglobin content may be due to both of these factors.

Foda, A. 1973. Changes in hematocrit and hemoglobin in Atlantic salmon (*Salar salar*) as a result of furunculosis disease. *J. Fish. Res. Bd. Can.* 30:467-468.

Comparative hematology studies were performed between health and bacterial-diseased juvenile salmon. In healthy fish the mean hematocrit value was 44.5% and the mean hemoglobin value was 9.5 g/100 ml of blood. In fish infected with *Aeramonas salmonicida*, which is the causative agent for furunculosis disease, the hematocrits and hemoglobin levels were significantly decreased. The changes in these blood parameters were attributed to hemorrhages caused by the infection.

Frankel, S., S. Reitman, and A. C. Sonnenwirth. 1970. *Gradwohl's Clinical Laboratory Methods and Diagnosis*, C. V. Mosby Co., Saint Louis, Missouri. Chap. 26.

Gardner, G. R. and P. P. Yevich. 1969. Studies on the blood morphology of three estuarine cyprinodontiform fishes. *J. Fish. Res. Bd. Can.* 26(2):433-447.

Blood samples were obtained during August from striped killifish (*Fundulus majalis*) and sheepshead minnow (*Cyprinodon variegatus*) at the site of capture in the field. Blood was taken from mummichogs (Fundulus heteroclitus) during all seasons in the field and from fish held in 2 types of holding aquaria for 1, 2, 3, 4, 15 and 30 days. Blood cell types observed in fresh and stained blood preparations consisted of erythrocytes, small and medium lymphocytes, eosinophils and thrombocytes. No basophils or neutrophils were observed.

Seasonal variations were found in the morphology of erythrocytes, eosinophils and thrombocytes in the blood of mummichogs. There was no significant difference between the differential counts of males and females and little overall variation was found in leucocytic cell type ratios. In addition, there were no significant differences in mean erythrocyte counts for the common mummichog during all sampling periods. There was a significant increase in the concentration of eosinophils in Fundulus heteroclitus kept in 20 liter aquariums over a 30-day period, when compared with fish held in 189-liter aquarium over the same time period. There was no change in lymphocyte or thrombocyte levels in fish held in 20-liter aquarium.

Gauldie, R. W., D. Purnell, and D. A. Robertson. 1977. Some biochemical similarities and differences between two jack mackeral species, Trachurus declivis and T. novaezelandiae. Comp. Biochem. Physiol. 58B:389-391.

Biochemical studies were done on two similar species of jack mackeral. Electrophoretic studies were performed on two loci each of hemoglobin, serum esterase and white and red muscle lactate dehydrogenase. Otolith iron was also compared in these two species. Both species had one identical locus and one unique locus for serum esterase and hemoglobin. Otolith iron was essentially identical for these species.

Giles, M. A. and W. E. Vanstone. 1976. Ontogenetic variation in the multiple hemoglobins of coho salmon (Oncorhynchus kisutch) and effect of environmental factors on their expression. J. Fish Res. Bd. Can. 33:1144-1149.

Hemoglobin polymorphism was examined in the blood of coho salmon at various stages of development by subjecting hemolyzates from the blood to micro-starch-gel electrophoresis. During the life cycle of coho salmon, three electrophoretically distinct hemoglobin patterns, which consisted of different combinations of 18 hemoglobin tetramers, were observed. The hemoglobins of embryos and yolk-sac alevins were composed of 1 cathodic and 12 anodic components. The hemoglobin pattern of fry consisted of three of the 12 anodic components. During smoltification, four new cathodic components were observed and the cathodic component and two of the anodic components, which were found in alevin and embryo hemolyzates, reappeared. There appeared to be no change in this latter hemoglobin pattern before the fish spawned and died.

Fry and presmolt coho salmon were exposed to variations in dissolved oxygen, temperature, and salinity. There appeared to be no significant modification of coho salmon hemoglobin polymorphs in direct response to these environmental conditions. In addition, the hematocrit levels of coho salmon fry and presmolts did not appear to be influenced by variations in temperature, dissolved oxygen or salinity.

Gordon, M. S. 1959. Ionic regulation in the brown trout (Salmo trutta L.) J. Exp. Biol. 36(2):227-252.

The effects of season and temperature (10° and 20°C) on osmotic and ionic regulations were investigated in brown trout subjected to stress similar to that encountered during migration from freshwater to seawater. Muscle and serum electrolyte levels, muscle total dissolved solids, and serum freezing point depression were determined. When trout were transferred from freshwater to 50% seawater at 20°C, blood concentrations rose significantly and then returned to normal in approximately one week. Similar responses were observed in trout transferred from 50% seawater to 100% seawater at 20°C. When trout were transferred from freshwater to 100% seawater at 20°C, serum concentrations were very high and mortality occurred. However, fish survived transfer from freshwater to full seawater at 10°C and serum concentrations returned to normal freshwater values within 10 days.

Seasonal differences were found in survival and regulatory abilities of brown trout. Seasonal changes in blood and muscle concentrations in trout within a given state of acclimation were also found. Variations in muscle concentrations paralleled blood changes. Variations in muscle concentrations were attributable to variations in intracellular water because the extracellular volume was constant.

Gordon, M. S., B. H. Amdur and P. F. Scholander. 1962. Freezing resistance in some northern fishes. Biol. Bull. 1972:5262.

The plasma osmolarity and chemical composition were examined in Labrador and New Brunswick fish. Short-horned sculpins and fjord cod, captured in Labrador during the early spring, were supercooled and had organic "antifreeze" compounds present in the blood. These antifreeze substances present in the blood of short-horned sculpin and fjord cod were not identified. The amounts of these compounds appeared to be variable. These sculpins and cods were very resistant to freezing when seeded with ice. When New Brunswick sculpin and tomcod (also captured in the early spring) were supercooled, they were not resistant to freezing and appeared to lack the "antifreeze" compounds.

Graham, T P. 1974. Chronic malnutrition in four species of sunfish in a thermally loaded impoundment. In: Gibbons, J. W. and R. R. Sharitz (eds.), Thermal Ecology Proceedings of a symposium held at Augusta, Georgia, May 3-5, 1973. U.S. Atomic Energy Commission, Oak Ridge, Tennessee, pp. 151-157.

The effects of sublethal thermal discharges on fish physiology was examined in four species of sunfish. Sunfish captured from a small cooling impoundment were visibly emaciated and had low condition factors. The emaciated specimens had low erythrocyte sedimentation rates, plasma iron, and hematocrit levels and high blood clotting times. Other symptoms of chronic malnutrition were also found. Malnutrition was attributed to elevated metabolic needs at high summer temperatures and to food deprivation resulting from a lack of food organisms at these elevated temperatures.

Haschemeyer, A.E.V. and W. Guschlbauer. 1977. Water binding by antifreeze glycoproteins from Antarctic fish. *Nature* 269:87-88.

Water binding by antifreeze glycoproteins was studied at subzero temperature in *Dissostichus mawsoni*, an Antarctic fish. Antifreeze glycoprotein solution at -5°C consisted of approximately 90% bulk water, 5% bound water, and 5% unfrozen water. Irrotationally bound water was estimated to be less than 0.1%.

Hattingh, J. and A.J.J. Van Pletzen. 1974. The influence of capture, and transportation on some blood parameters of freshwater fish. *Comp. Biochem. Physiol.* 49A:607-609.

Hematology was studied in mudfish after capture and transportation to the laboratory. Decreased hemoglobin, hematocrit, plasma osmolarity, blood pH, specific gravity, erythrocyte and leucocyte counts and plasma protein levels and increased plasma and cell water content, erythrocyte fragility, thrombocyte counts, and methemoglobin levels were found within 2-3 days after capture and transportation. All variables returned to normal within 7-10 days after capture and transportation of the fish. The author suggested that hematological studies should not be started until several days after capture and transportation.

Haws, T. G. and C. J. Goodnight. 1962. Some aspects of the hematology of the two species of catfish in relation to their habitats. *Physiol. Zool.* 35(1):817.

Some aspects of the hematology of brown bullhead and channel catfish were compared. Similar blood pH, specific gravity, hematocrit, hemoglobin and oxygen capacity levels were found for both species. Channel catfish had significantly higher erythrocyte counts than the bullhead. Erythrocyte size was larger in the bullheads than in catfish, although the shape of the cells were similar. Positive correlations were found between weight and hematocrit and between weight and erythrocyte counts. Decreases in hematocrit and erythrocyte counts were correlated with spawning condition. Oxygen and carbon dioxide dissociation curves were examined for both species.

Hesser, E. F. 1960. Methods for routine fish hematology. *Prog. Fish-Cult.* 221:164-171.

Routine techniques for studying fish hematology were reviewed and evaluated. Enumeration of leucocytes and erythrocytes were facilitated using Shaw's solution. The hemal stain was used in preparation of dry smears for blood cell differential counts. Standard methods for the determination of microhematocrit and hemoglobin levels were also discussed.

Hickman, C. P., Jr., R. A. McNabb, J. S. Nelson, E. D. Van Breeman and D. Comfort. 1964. Effect of cold acclimation on electrolyte distribution in rainbow trout (*Salmo gairdneri*). *Can. J. Zool.* 42:577-597.

The effect of acclimation to constant low temperatures on water and electrolyte regulation in tissue and blood of 2-year-old rainbow trout was studied. When trout acclimated to 16°C were exposed to 6°C, periodic measurements were made of plasma, muscle and brain sodium, potassium, and chloride levels and plasma calcium, magnesium and phosphate levels. During the first days of exposure to cold, there was a significant drop in muscle, brain, and plasma water content, accompanied by an increase in total body weight. There was no significant change in chloride space or chloride-potassium space estimates during cold acclimation. There were significant transient variations in concentrations of many plasma electrolytes.

There were significant reciprocal changes in brain, sodium and potassium levels shortly after transfer to 6°C, and significant increases in brain chloride levels after 3 weeks exposure to 6°C. During cold exposure there were significant but mostly transient variations in muscle potassium and sodium levels.

Houston, A. H. 1973. Environmental temperature and the body fluid system of the teleost. In: Chauvin, W. (ed.). *Responses of Fish to Environmental Changes*, Charles C. Thomas, Publisher, Springfield, Illinois, pp. 87-162.

Review of the effects of temperature on water and electrolyte balance in teleosts.

Houston, A. H. and D. Cyr. 1974. Thermoacclimatory variation in the haemoglobin systems of goldfish (*Carassius auratus*) and rainbow trout (*Salmo gairdneri*). *J. Exp. Biol.* 61:455-461.

Acclimation of rainbow trout and goldfish to elevated temperatures resulted in significant increases in hematocrit and hemoglobin levels. Two hemoglobin components were present in goldfish acclimated to 2°C, while three components were present in fish acclimated to 20° and 35°C. Trout acclimated to 2°, 10°, and 18°C had nine hemoglobin variants. The data supports the theory that specific hemoglobin functions are altered during the thermoacclimatory process.

Houston, A. H. and M. A. DeWilde. 1968. Thermoacclimatory variations in the haematology of the common carp, *Cyprinus carpio*. *J. Exp. Biol.* 49:71-81.

> The influence of temperature on the hematology of winter, summer, and autumn populations of carp was investigated. There was a direct relationship found between temperature and erythrocyte number, hematocrit, and hemoglobin levels. There was an increase in mean erythrocyte volume at low temperatures. No change was found in mean erythrocyte volume, mean erythrocytic hemoglobin, and mean erythrocyte hemoglobin concentration in goldfish exposed to high temperatures. Possible forms of cardiovascular-respiratory adjustments in fish were discussed.

Houston, A. H. and M. A. DeWilde. 1969. Environmental temperature and the body fluid system of the freshwater teleost. III. Hematology and blood volume of thermally acclimated brook trout, *Salvelinus fontinalis*. *Comp. Biochem. Physiol.* 28:877-885.

> Changes in blood volume and hematology were associated with acclimation of brook trout to different temperatures (2°, 5°, 8°, 10°, and 20°C). Hematocrit, hemoglobin, erythrocyte counts, and plasma and blood volume increased with elevated temperature. Mean erythrocyte hemoglobin appeared to be stable over the temperature ranges examined.

Houston, A. H. and J. A. Madden. 1968. Environmental temperature and plasma electrolyte regulation in the carp, *Cyprinus carpio*. *Nature* 217:969-970.

> Plasma samples were analyzed in carp acclimated to 4°, 17°, 27°, and 33°C. Mean plasma chloride and calcium levels increased with temperature. Plasma phosphate levels increased markedly between 4° and 17°C, and decreased slightly at the higher temperatures. Plasma sodium values were highest at 27°C, being 6-7% greater than all other groups. There was no consistent trend in plasma potassium or magnesium concentrations with temperature. Although plasma osmolarity decreased at elevated temperatures, the sum of all cations plus chloride increased. The data suggested that the percentage of total plasma osmolarity attributed to a nonelectrolyte increased at low temperatures. Total plasma protein levels indicated that this nonelectrolyte was not protein.

Houston, A. H., J. A. Madden and M. A. DeWilde. 1970. Environmental temperature and the body fluid system of the freshwater teleost. IV. Water-electrolyte regulation in thermally acclimated carp, *Cyprinus carpio*. *Comp. Biochem. Physiol.* 34:805-818.

> Muscle and blood chemistry were examined in summer, fall, and winter carp, acclimated to temperatures ranging from 2°C to 27°C. Body fluid electrolytes were requested over a wide range of temperatures. There were significant seasonal changes in plasma ions. There was a progressive decrease in fish muscle water content from summer to winter. Muscle chloride,

calcium, and magnesium were relatively constant, while muscle sodium and potassium appeared to vary with season and temperature.

Houston, A. H., J. A. Madden, R. J. Woods and H. M. Miles. 1971. Some physiological effects of handling and tricaine methanesulphonate anesthetization upon the Brook trout, Salvelinus fontinalis. J. Fish. Res. Bd. Can. 28(5):625-633.

The effects of handling and anesthetization on hematology were investigated in brook trout. Anesthetization resulted in elevated hematocrit, hemoglobin, and total dissolved solid levels, suggesting that hemoconcentration occurred. Tissue water content increased, and tissue and plasma electrolytes (sodium, chloride, calcium, and magnesium) levels were also altered. Blood pressure and ventilatory activity decreased, whereas heart rate increased within 30 seconds of exposure to anesthesia. Significant variations in plasma glucose and lactate and some electrolyte levels occurred in response to handling alone.

Houston, A. H., R. S. Reaves, J. A. Madden, and M. A. DeWilde. 1968. Environmental temperature and the body fluid system of the freshwater teleost. I. Ionic regulation in thermally acclimated rainbow trout, Salmo gairdneri. Comp. Biochem. Physiol. 25:563-581.

The effect of environmental temperature (3-21°C) on ionic regulation was studied in fall-winter and summer rainbow trout. Both summer and fall-winter trout had relatively stable plasma sodium and chloride levels. No significant differences were found in the plasma potassium levels of summer trout, except at 17°C. Levels of plasma calcium, potassium, magnesium, and phosphate increased with temperature in fall-winter trout. Tissue water levels were decreased at high temperatures. No consistent relationship was found between temperature and water distribution in summer trout. However, among the fall-winter trout, the cell phase volume was inversely and the extracellular space was directly related to temperature. Seasonal variations were found in plasma potassium, sodium and chloride levels, tissue potassium and chloride levels, and water distribution.

Jones, R. M. 1967. McClung's Handbook of Microscopical Technique. Hafner Publishing Co., New York. 790 p.

Kamra, S. K. 1966. Effect of starvation and refeeding on some liver and blood constituents of Atlantic Cod (Gadus morhua L.) J. Fish Res. Bd. Can. 23(7):975-982.

Starvation effects on components of the liver and blood were investigated in serially sampled Atlantic cod (Gadus morhua L.) Body weight, liver weight, and liver glycogen content decreased during starvation, but returned to normal within 2 weeks after feeding was resumed. Transient increases in liver weight and liver glycogen content were found during liver regeneration. Mean plasma protein, glucose, and hematocrit levels decreased initially during fasting. Prolonged starvation, however, resulted in increased protein, glucose, and hematocrit levels, possibly due to plasma dehydration. Plasma pH increased during starvation and returned to normal after feeding was resumed. No consistent trend was found between plasma free polysaccharide content and starvation or feeding.

Kaplan, M.G.D. 1971. The effects of sewage on the hematology of *Gasterosteus aculeatus*. MS Thesis, San Francisco State College, San Francisco, California. 125 p.

Leach, G. L. and M. H. Taylor. 1976. Seasonal measurements of serum glucose and serum cortisol in a natural population of *Fundulus heteroclitus*. *Comp. Biochem. Physiol.* 56A:217-223.

Seasonal effects on serum glucose levels of field populations of *Fundulus heteroclitus* were found. One peak in serum glucose levels, which was inversely correlated with temperature, occurred between September to mid-May. A second peak in glucose values, which was correlated with spawning season, occurred during June and July. No consistent seasonal effects were found for serum cortisol values of killifish. No significant differences in serum glucose or cortisol values were found between males and females.

Leatherland, J. F., C. Y. Cho, and S. J. Slinger. 1977. Effects of diet, ambient temperature, and holding conditions on plasma thyroxine levels in rainbow trout (*Salmo gairdneri*). *J. Fish. Res. Bd. Can.* 34:677-682.

The plasma thyroxine levels were measured in rainbow trout subjected to different combinations of diet, temperature and holding conditions. Similar plasma thyroxine levels were found in rainbow trout acclimated to 19°C between rainbow trout fed a diet of 6% soybean oil and trout fed a diet of 6% tallow fat. However, in rainbow trout acclimated to 7°C, higher thyroxine levels were found in trout fed the soybean diet than fish fed the tallow diet. Plasma thyroxine levels of fish fed the soybean diet were greater in trout acclimated to 7°C than in trout acclimated to 19°C. There were no apparent temperature related variations in thyroxine levels of fish fed the tallow fat diet. The plasma thyroxine levels were lower in fish starved for 40 days than in fish fed daily. Photoperiod and light limitation had no significant effect on plasma thyroxine concentrations.

Lewis, W. M. (1963) Maintaining Fishes for Experimental and Instructional Purposes. Southern University Press, Carbondale, Illinois. 95 pp.

Lieb, J. R., G. M. Slane, and C. G. Wilber. 1953. Hematological studies on Alaskan fish. *Trans. Am. Microscop. Soc.* 72(1):37-47.

Blood samples from Thymallus signifer and Cristivomer namaycush were examined to determine blood cell composition and morphology. Lymphocytes were the most abundant leucocyte in blood of lake trout and grayling, constituting 91% and 87% of the total leucocytes, respectively. Monocytes comprised 8.2% and neutrophils 0.6% of the total leucocytes in lake trout, while monocytes comprised 1%, neutrophils 5%, lymphoid hemoblasts 4%, and proerythrocytes 3% of the total leucocytes present in grayling.

The mean cell diameters of the cell types present in lake trout were 17.8 x 10.35 μ for erythrocytes, 8.60 μ for small lymphocytes, 10.46 μ for large lymphocytes, 12.92 μ for monocytes, and 12.75 μ for neutrophils. Morphology of the cell types present in lake trout and grayling were discussed.

Mackay, W. C. 1974. Effect of temperature on osmotic and ionic regulation in goldfish, Carassius auratus L. J. Comp. Physiol. 88:1-19.

The effect of temperature on osmotic and ionic balance was studied in goldfish, Carassius auratus. When fish were subjected to acute temperature increases, urine flow was increased. Urine flow appeared to show temperature acclimation. The urine flow of fish acclimated to 10°C and measured at 20°C was much greater than the urine flow of fish acclimated to 30°C and measured at 20°C. Over the range of 14° to 24°C, the renal reabsorption of sodium and chloride of 24°C-acclimated fish appeared to be independent of temperature, but at lower temperatures (6.5°C) reabsorption was inhibited. Acute temperature variation between 6.5° and 24°C did not alter the water permeability of the renal tubules. Temperature compensation was found for urine osmolarity, sodium, potassium and chloride levels of goldfish acclimated to 10°C and 30°C. The renal excretion rates of sodium and chloride, measured at 20°C, were significantly greater in fish acclimated to 10°C than in fish acclimated to 30°C, indicating temperature acclimation. When measured at the acclimation temperature, the sodium and chloride excretion rates of fish acclimated to 30°C were greater than those of fish acclimated to 10°C. The branchial sodium uptake of fish acclimated to 10°, 20°, and 30°C in demineralized water, increased with expsoure to acute temperature increases in tapwater. Comparison of the three groups at 20°C, revealed that the net sodium uptake rate was highest in fish acclimated to 10°C and lowest in fish acclimated to 30°C. The temperature acclimation of sodium uptake appeared to be correlated with variations in plasma sodium levels of the three groups. Correlations were also found between temperature acclimation and plasma chloride levels of fish acclimated in demineralized water, but the net chloride uptake rate was much less than the net sodium uptake rate. Variable levels of sodium and chloride uptake were found in fish acclimated in tapwater. Temperature acclimation was not found for sodium and chloride uptake in fish held in tapwater. Comparison of the uptake and excretion of electrolytes of fish acclimated in tapwater indicated that the fish were in salt balance.

Mairs, D. F. and T. E. Kennedy. 1962. Evaluations of some heparinized capillaries for microhematocrit determinations of warmwater fishes. *Prog. Fish-Cult.* 24(4):188-189.

Commercially-prepared heparinized microhematocrit tubes were evaluated using blood of 14 species of warmwater fishes. Tubes were considered to be unsatisfactory for hematocrit determinations if there was blood clot formation in the tube. The commercially prepared tubes were satisfactory for hematocrit determinations on bluegills only.

McCarthy, D. H., J. P. Stevenson, and M. S. Roberts. 1973. Some blood parameters of the rainbow trout (*Salmo gairdneri* Richardson). I. The Kamloops variety. *J. Fish Biol.* 5:1-8.

The hematology of normal fourteen-month old rainbow trout of the Kamloops strain was examined. Fish were held under natural hatchery conditions and fed a uniform diet. Values were given for erythrocyte sedimentation rate, hemoglobin, hematocrit, erythrocyte count, erythrocyte cell length and width, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, leucocyte count, differential leucocyte count, plasma total protein, and plasma glucose. The results were compared with published literature for Idaho and Shasta strains of rainbow trout and the differences between these studies were discussed.

McKnight, I. M. 1966. A hematological study of the mountain whitefish, *Prosopium williamsoni*. *J. Fish. Res. Bd. Can.* 23(1):45-63.

Baseline hematology values for mountain whitefish were measured over a one-year period. The parameters measured included hemoglobin, hematocrit, erythrocyte, thrombocyte and leucocyte counts, erythrocyte cell length and width, and leucocyte differential counts. Differences in hematological values associated with age, sex, or season were discussed.

McLeay, D. J. 1975. Sensitivity of blood cell counts in juvenile coho salmon (*Oncorhynchus kisutch*) to stresses including sublethal concentrations of pulpmill effluent and zinc. *J. Fish. Res. Bd. Can.* 32:2357-2364.

When fish were transferred from optimal conditions to high temperature and crowded holding conditions, there was a marked decrease in white blood cell-thrombocyte counts at 12-96 hours of exposure. Erythrocyte counts increased only slightly at 12 and 48 hours. During a 28-day acclimation period after transfer to simulated stress conditions, there was an initial decrease in leucocyte-thrombocyte counts, followed by a return to normal within 2-4 days, whereas there was no significant variation in erythrocyte counts.

The transfer of acclimatized fish to a high sublethal level of pulpmill effluent for a 96-hour period resulted in a decline in the leucocyte-thrombocyte counts which was greatest at 24 hours. This was accompanied by a significant decrease in erythrocyte counts at 24 hours.

The leucocyte-thrombocyte counts were significantly reduced at concentrations between 0.2 and 0.9 of the 96-hour LC50 level. When foam fractionation was used to detoxify pulpmill effluent, a 24-hour exposure to 15% vol/vol of the effluent resulted in less alteration in the leucocyte-thrombocyte counts.

When acclimitized fish were exposed to sublethal level of zinc, there was a decrease in leucocyte-thrombocyte counts proportional to the concentration, although differences were significant only at 0.5 LC50 levels and above. There were no significant differences in erythrocyte counts.

The leucocyte-thrombocyte counts in salmon blood provide a rapid and sensitive method for monitoring pulpmill effluent stress.

Meisner, H. M. and C. P. Hickman, Jr. 1962. Effect of temperature and photoperiod on the serum proteins of the rainbow trout, Salmo gairdneri. Can. J. Zool. 40(2):127-130.

The effect of different combinations of temperatures (8° and $16^{\circ}C$) and photoperiod (16 and 8 hours of light daily) on the serum protein of Salmo gairdneri were investigated. There was no significant change in serum proteins associated with photoperiod. Trout acclimated to $8^{\circ}C$ had significantly larger albumin/globulin ratios than fish acclimated to $16^{\circ}C$.

Mertz, E. T. 1969. Amino acid and protein requirements of fish. In: Neuhaus, O. W. and J. E. Halver (eds.). Fish in Research, Academic Press, New York/London, pp. 233-241.

Amino acid and protein requirements were studied in fish. Fish and young mammals had similar amino acid requirements, whereas fish protein requirements were greater than twice those of young mammals or birds and appeared to increase with temperature. Fingerling salmon, and possibly other fish, had a high organic requirement, which was similar to the percentage of dietary protein required by birds. Mammals and birds had higher tryptophan and isoleucine requirements than fish, whereas other amino acid requirements were similar in fish and mammals. It was suggested that higher plasma amino acid levels in fish may result in an increased utilization of dietary protein for synthesis as compared with other animals. Plasma amino acids may exist in conjunction with elevated levels of enzymes and nucleic acids involved in intracellular protein synthesis. It was also suggested that enzymes involved in the deanimation of amino acids may be present in lower levels in fish than in mammals or birds.

Murray, S. A. and C. G. Burton. 1977. Consolidation of baseline information, development of methodology, and investigation of thermal impacts on freshwater shellfish, insects and other biota. Task 3: Biochemical methodology, aquatic thermal impacts. TVA Internal Report No. I-WQ-77-3.

Routine methods of blood sampling and analysis in Carassius auratus were discussed. Hematological techniques reported in this article include methods for the determination of erythrocyte sedimentation rate, hematocrit, hemoglobin, plasma osmolarity, and blood cell enumeration, differentials and morphology.

Murray, S. A., and C. G. Burton. 1979. Density effects on goldfish blood. II. Cell morphology. Comp. Biochem. Physiol. 62A:559-562.

The effect of loading density (0.8 and 4.19 fish centimeters/liter of water) on blood cell morphology and abundance was studied in goldfish. Goldfish held at the higher loading density had significantly lower numbers of erythrocytes and thrombocytes, and higher numbers of lymphocytes and total leucocytes. Erythrocyte size was smaller in fish held at the higher density.

Murray, S. A. 1980. Effects of loading density on catfish blood. Experientia 36:205-206.

Nordlie, F. G. 1976. Influence of environmental temperature on plasma ionic and osmotic concentrations in Mugil cephalus Lin. Comp. Biochem. Physiol. 55A:379-381.

The effect of temperature on plasma ionic and osmotic regulation was studied in striped mullet. When acclimation temperatures increased from 10° to 30°C, the plasma osmolarity, sodium and chloride levels in striped mullet increased, whereas there was no significant change in plasma potassium levels.

Normandeau, D. A. 1962. Microhematocrit values for some salmonids reared in New Hampshire. Prog. Fish-Cult. 24(4):172-176.

Hematocrit values were determined for immature brook trout, rainbow trout, lake trout, splake trout, and landlocked salmon. All blood samples were collected between June and September, when water temperatures ranged from 47°-62°F.

There were significant differences between sampling stations throughout the state, but these differences were not consistent. There appeared to be no correlation between the hematocrit and water temperatures, but significant correlations were found between hematocrit and sampling time. The differences may also be associated with age or other factors.

Norton, V.A.M. 1975. Osmoregulation in the channel catfish Ictalurus punctatus (Rafinesque). Dissertation, Memphis State University, 1975.

The effect of hypophysectomy on ionic and osmotic regulation was studied in channel catfish held at 23° and 30°C. Salinity effects on these regulatory systems were also examined in intact catfish. When environmental ion levels were hyperionic to normal plasma ion levels and catfish acclimated to 23°C in freshwater were transferred to saltwater, the plasma sodium and chloride levels were elevated. When freshwater fish acclimated to 23°C were transferred to hyperionic saltwater, acclimation took 15 days. However, in isoionic saltwater there was no variation in plasma ion levels. At 23°C, fish acclimated to isoionic saltwater and those acclimated to freshwater had similar plasma sodium and chloride values upon transfer to a hypersaline environment. Transfer of fish from saltwater to freshwater resulted in a significant reduction in plasma sodium and chloride levels. Handling did not significantly alter plasma chloride and sodium levels of freshwater catfish acclimated to 23°C.

Hypophysectomized channel catfish survived in 21° to 31°C freshwater. Hypophysectomized fish held at 23°C had lower plasma chloride and sodium levels than normal freshwater fish. However, longterm hypophysectomized fish held at 30°C appeared to recover completely. Although permanent damages were not found in urinary sodium and chloride excretion or urine volume of hypophysectomized catfish, there were differences in the urinary excretion patterns between longterm hypophysectomized and intact fish.

A model of the osmoregulatory capabilities of channel catfish was proposed.

Parry, G. 1961. Osmotic and Ionic changes in blood and muscle of migrating salmonids. J. Exp. Biol. 38:411-427.

Ionic and osmotic regulation were studied in the blood and tissue of migrating salmonids during different stages of the life cycle. Muscle water content, muscle and plasma electrolyte levels, and the plasma freezing-point measurements were used to determine the regulatory abilities of *Salmo salar*. When smolts were transferred from freshwater to seawater, blood concentration increased by approximately 12%. Transfer of adult fish from seawater to freshwater resulted in a 5% decrease in blood concentration. Changes in muscle water content and electrolytes suggested that transfer of fish from one salinity to another may cause variation in the extracellular space.

Smolts and fresh-run adult salmon appeared to have good osmotic regulation, while kelts did not appear to osmoregulate without difficulty.

Pearcy, W. G. 1961. Seasonal changes in osmotic pressure of flounder sera. Science 134:193-194.

Seasonal variations in the serum osmolarity was investigated in Pseudopleuronectes americanus (Walbaum). The mean freezing point depression of sera during the winter was 1.15°C, whereas it was 0.63°C during the summer. Although sodium and chloride concentrations increased during the winter, these ions accounted for only part of the seasonal increase in serum osmolarity. Other substances were responsible for most of the increased serum osmolarity during the winter.

Phillips, A. M., Jr. 1961. Effect of water temperature and diet on the blood glucose of brook trout. Prog. Fish-Cult. 23(2):66-69.

The effects of diet (temporary starvation, meat diet, and meat-meal diet) and water temperature (42°F and 52°-59°F) on the blood glucose levels of brook trout were studied. Temperature had no significant effect on blood glucose levels of temporarily starved or meat-fed trout. However, meat-meal fed trout at 52°-59°F had significantly higher blood glucose levels than those at 42°F. Temporary starvation did not appear to affect blood glucose levels. Trout fed the meat diet had significantly lower glucose levels than controls, while those fed the meat-meal diet had significantly higher glucose levels.

Pickford, G. E., A. K. Srivastava, A. M. Slicher, and P. K. Pang. 1971. The stress response in the abundance of circulating leucocytes in the killifish, Fundulus heteroclitus. 1. The cold-shock sequence and the effects of hypophysectomy. J. Exp. Zool. 177:89-96.

When male killifish were exposed to 3-minute cold shock and then returned to 20°C, hematology studies during the 2-hour recovery period revealed an alternating sequence of leucopenia and leucocytosis. This was not attributable to changes in abundance of any particular leucocytic cell type. No significant differences in erythrocyte numbers were found between cold shocked and control killifish. Serum chloride levels, which were not correlated with leucocytosis or leucopenia, were significantly increased in cold shocked fish. Serum glucose levels of cold shock fish were significantly elevated at 2 hours post-coldshock.

Exposure of hypophysectomized killifish to cold shock confirmed the above post-shock results, but hypophysectomy minimized or abolished the second leucocytic phase.

Piper, R. G. and R. F. Stephens. 1962. A comparative study of the blood of wild and hatchery-reared lake trout. Prog. Fish-Cult 24(2):81-84.

Comparative hematological studies were performed on hatchery-reared and wild Salvelinus namaycush. There were no significant differences in hemoglobin level and erythrocyte numbers between wild and hatchery-reared trout. Normal mean values for erythrocyte numbers were 800,000 cells/mm^3 and for hemoglobin 6.1

g/100 ml of blood. The normal mean hematocrit level in hatchery-reared trout was 29.7%. Hatchery-reared trout had slightly higher hematocrit levels and erythrocyte numbers in September than in March.

Platner, W. S. 1950. Effects of low temperature on magnesium content of blood, body fluids and tissues of goldfish and turtles. Am. Journ. of Physiol. 161:399-405.

Goldfish exposed to 0^o-1^oC for 60 hours showed an increase in serum magnesium and a decrease in hematocrit levels and red blood cell numbers. An increase in serum magnesium was also found in goldfish exposed to cold and anoxia.

When turtles were exposed to $2^o \pm 3^oC$ for 11 days, there was an increase in serum magnesium, while there was no change in hematocrit or erythrocyte numbers. Magnesium also increased in the perivisceral fluid, decreased in muscle and skin, and remained almost constant in erythrocytes.

Poluhowich, J. J., and R. P. Parks. 1972. A comparative study of blood chemistry and respiration in marine and freshwater eels. Prog. Fish-Cult. 34:33-38.

Powers, D. A. 1974. Structure, function, and molecular ecology of fish hemoglobins. Annals of the New York Academy of Science. 241:472-490.

The structure, function, and molecular ecology of hemoglobin was examined in two subgenera (Catostomus and Pantosteus) of Catostomid fishes acclimated to temperatures ranging from 2^o to 30^oC. Cathodal hemoglobin components were present in Pantosteus but not in Catostomus. Catostomid fish apparently used some mechanisms to facilitate oxygen binding and oxygen release to tissues when temperatures were increased. Elevated hematocrit levels with stable mean erythrocytic hemoglobin concentrations resulted in elevated hemoglobin levels at high temperatures. In addition, the decrease in erythrocytic ATP at increased temperature may have resulted in increased oxygen affinity. The maximum of subunit cooperativity was shifted to a lower pH value possibly resulting from decreased ATP levels. The blood pH value was decreased to correspond with some physiological parameters.

Powers, D. A. and A. B. Edmundson. 1972. Multiple hemoglobins of Catostomid fish. I. Isolation and characterization of the isohemoglobins from Catostomus clarkii. J. Biol. Chem. 247(20):6686-6693.

Multiple hemoglobins were studied in Catostomus clarkii. Hemoglobins were separated, isolated, and characterized.

Prosser, C. L., W. Mackay and K. Kato. 1970. Osmotic and ionic concentrations in some Alaskan fish and goldfish from different temperatures. Physiol. Zool. 43(2):81-89.

Ionic and osmotic concentrations of the muscle and plasma of some Alaskan marine fish were examined after capture at different depths and temperatures. The ion concentrations, water content, and extracellular space were studied in the serum and muscle of goldfish acclimated to 5, 15, and 25°C for 3 weeks.

Generally, fish species with reduced plasma osmolarity values were captured above or near the thermocline (9°-10°C), whereas those with increased values were captured from the colder water (5°-7°C). The change in plasma osmotic concentrations appeared to be due to changes in sodium and chloride concentrations. Muscle sodium and potassium concentrations were generally higher in species with elevated plasma osmolarity.

Plasma electrolyte levels in goldfish were significantly lower at 5°C than at 15° or 25°C. Muscle potassium was significantly lower at 5°C than at 25°C, although muscle sodium levels were not significantly changed.

Rao, G. M. 1969. Effect of activity, salinity, and temperature on plasma concentrations of rainbow trout. Can. J. Zool. 47:131-134.

Rainbow trout were subjected to different combinations of salinity, activity, and temperature conditions. The plasma was nearly isomotic with water at 7.5% salinity. Plasma osmolarity values were higher in exercised fish than in unexercised fish. There was no significant difference in chloride concentrations between exercised and resting fish. Plasma osmolarity and chloride concentrations were higher in fish exposed to 5°C than those exposed to 15°C.

Reaves, R. S., A. H. Houston, and J. A. Madden. 1968. Environmental temperature and the body fluid system of the fresh-water teleost. II. Ionic regulation in rainbow trout, *Salmo gairdneri*, following abrupt thermal shock. Comp. Biochem. Physiol. 25:849-860.

When 11°C-acclimated rainbow trout were subjected to moderate (+5°C, -6°C) and acute (+10°C, -10.5°C) temperature shock, there were changes in plasma and tissue ionic regulations. Responses of moderate and acute cold- and heat-shocked trout were characterized by decreased tissue water content, increased extracellular fluid volume, and an initial reduction in plasma sodium and chloride concentrations. These results suggested that goldfish had, in part, a somewhat generalized temperature stress response, which was not dependent on magnitude or direction of the change in temperature.

Acute temperature shock resulted in abrupt changes in phase volume, while moderate shock resulted in more gradual changes in phase volume of equivalent magnitude. In addition, there were differences in the effects of heat and cold shock on the plasma and tissue electrolytes. The results indicated that specific effects of moderate and abrupt cold and heat shock were superimposed on the generalized temperature response.

Ridgeway, G. J. 1969. Blood groups in salmonid fishes. In: Neuhaus, O. W. and J. E. Halver (eds.). Fish in Research. Academic Press, New York/London/1969. pp. 185-193.

The blood groups of healthy salmonid fishes were studied. Literature on blood typing in salmonid fishes was reviewed. To determine intraspecific antigenic differences, rainbow trout and sockeye salmon were isoimmunized periodically. Isoagglutinins of rainbow trout were usually found within 9 weeks of the initial isoimmunization. The agglutinin titers of isoimmunized trout sera ranged from 1:2 to 1:256. Isoimmunization of sockeye salmon revealed some individual antigenic differences. The most complex system of blood groups in sockeye salmon and rainbow trout was the B-system. The B-system consisted of sets of interrelated antigens, which included the phenogroups ABHJ, HJM, FHJLM, CHJM, and CHJ. Experimental breeding of rainbow trout revealed that some groups of reactions (ABHJM, CHJ, CJ, and HJ) were inherited as units. There were differences in the frequency of the blood groups among geographic races or subpopulations of sockeye salmon.

Ridgway, G. J. 1956. Some cytological observations on fish erythrocytes. Prog. Fish-Cult. 18:67-69.

Mitochondria and reticular material were found in the erythrocytes of fingerling and adult fall Orcorhynchus tshawytacha, and adult Orcorhynchus nerka and Salmo gairdneri. The presence of mitochondria and reticular material in fish erythrocytes did not appear to be a good indicator of anemia.

Sabnis, P. B. and P. V. Rangnekar. 1962. Blood cell formation in the freshwater teleost, Ophiocephalus punctatus (Bloch). Journal of Anim. Morph. Physiol. 9(2):124-130.

Blood and tissue samples were examined in specimens of Ophiocephalus punctatus within 48 hours of capture. Blood cells observed were erythrocytes, thrombocytes, lymhocytes (small and medium) and fine (acidophilic and basophilic) granulocytes. Thrombocytes were spherical and had a tendency towards clumping. Although no coarse granulocytes were present in the blood, they were present in kidneys, spleen, and other tissues. The intertubular tissue of the kidneys was primarily hemopoietic, while the spleen appeared to assume a secondary role in hemopoiesis.

Saunders, D. C. 1968. Differential blood cell counts on 50 species of fishes from the Red Sea. Copeia 3:491-498.

Differential blood cell counts and erythrocyte size measurements were made on blood from 50 species of fish collected from the Red Sea during a ten-month period. Blood cell types found in the elasmobranch were monocytes, thrombocytes, eosinophils, heterophils, and erythrocytes. No hemocytoblasts, lymphocytes nor neutrophils were found in the species examined.

In teleostean species hemoblasts, macrophages, lymphocytes, thrombocytes, eosinophils, neutrophils and erythrocytes were present, while no basophils were found. Thrombocytes and lymphocytes were the two most predominant leucoytic cell types present in the circulating blood of teleostean species. The range of mean erythrocyte length was 7.5 to 12.2 μ and of mean erythrocyte width was 6.0 to 9.5 μ in blood from teleosts. The mean erythrocyte length was 17.5 μ and the mean erythrocyte width was 12.5 μ in blood of elasmobranchs.

Saunders, D. C. 1967. Neutrophils and Arneth counts from some Red Sea fishes. Copeia 3:681-683.

Blood was obtained from 9 species from the Serranidae family and one species from the Parapercidae family. Granulocytes were examined and Arneth counts performed on the first 100 granulocytes of all types seen on an individual blood smear. Because no basophils and only one eosinophil were found in the blood smears, all Arneth counts were performed on neutrophils, with the exception of the one eosinophil. The mean counts ranged from 1.04 to 1.65.

Saunders, D. C. 1966. Differential blood cell counts of 121 species of marine fishes of Puerto Rico. Trans. Amer. Microsc. Soc. 85(3):427-449.

Differential blood cell counts and erythrocyte size measurements were made on blood from 121 species of fishes collected from the coasts of Puerto Rico during January through April. The cell types present in the blood of the elasmobranchs were erythrocyte hemocytoblasts, monocytes, lymphocytes, thrombocytes, eosinophils, neutrophils, heterophils and macrophages. The cell types present in the blood of teleosts were erythrocytes, hemoblasts, thrombocytes, lymphocytes, eosinophils, basophils, neutrophils, ring cells, and macrophages. The mean percentage of each leucocytic cell type present was determined for each species. Thrombocytes were the most predominant leucocyte present and lymphocytes were the next. The species mean erythrocyte length ranged from 7.2 to 17.0 μ in teleosts and from 17.3 to 22.5 μ in elasmobranchs and the species mean erythrocyte width ranged from 5.3 to 10.0 μ in teleosts and from 12.6 to 16.0 μ in elasmobranchs. Erythrocyte counts on 23 species of teleosts were made using a Neubauer hemocytometer. The species mean erythrocyte counts ranged from 2,270,000 to 6,480,000 cells/mm^3.

Schiffman, R. H. 1959. Method for repeated sampling of trout blood. Prog. Fish-Cult. 21(4):151-153.

Two methods for repeated sampling of anesthetized fish were discussed. In the first method, a needle was inserted into the dorsal aorta through the roof of the mouth in large fish. Another method of sampling blood from the dorsal aorta is to insert the needle anterior to the first gill arch. This method may be used for small fish.

Scholander, P. F., L. von Bam, J. W. Kanwisher, H. T. Hammel and M. S. Gordon. 1957. Supercooling and osmoregulation in Artic fish. *J. Cell Comp. Physiol.* 49:5-24.

> The plasma freezing point was examined in invertebrates and fishes living in subzero seawater (-1.7° to -1.8°C). Seeding both deepwater and surface water fishes with ice crystals resulted in freezing and death. The deepwater fish, which seldom come in contact with ice, had a plasma freezing point depression of 0.9° to -1.0°C in the summer. The surface water fishes had a plasma freezing point depression of approximately -0.80°C in the summer and approximately 1.50°C in the winter. Shallow water fish, which came in contact with the ice in the winter, prevented freezing by increasing the plasma osmotic concentration to a level which was almost isosmotic with seawater. The invertebrates examined were also almost isosomotic with seawater.

Schreck, C. B., R. A. Whaley, M. L. Bass, O. E. Maughan, and M. Solazzi. 1976. Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. *J. Fish. Res. Bd. Can.* 33:76-84.

> Exposure of rainbow trout to electroshock resulted in elevated plasma corticoid levels, lactate levels, and thrombocyte: leucocyte ratios immediately and elevated glucose levels within 3 hours post shock. No significant variations in hematocrit plasma protein, calcium, magnesium or androgen levels were found. Plasma lactate concentrations returned to normal within 3 hours, while glucose and corticoid levels remained high for at least 6 hours. Respiratory and cardiac activity appeared to be altered immediately after electroshocking. No significant differences in 13 isoenzymes from plasma, liver, and muscle were found between fish captured by electrofishing and those captured by dipnet. The data from this study suggested that at least 6 hours were required for fish to return to preshock conditions.

Shell, E. W. 1961. Chemical composition of blood of smallmouth bass. *United States Department of the Interior Fish and Wildlife Service Bureau of Sport Fisheries and Wildlife Research Report S7 Published by the U.S. Fish and Wildlife Service, Washington*. 1961:1-36.

> The chemical composition of the blood of smallmouth bass was examined at 3 to 4 week intervals from June through November 1958. The parameters measured included serum sodium, potassium, calcium, chloride, total protein, globulin, albumin, albumin-globulin ratio, total phosphorus, lipid phosphorus, inorganic phosphorus, cholesterol, total nonprotein nitrogen, uric acid, and blood creatinine, glucose, and iron. Cylical variations with respect to time were found in 13 of these chemical components. Many components decreased initially and then increased during the rest of the experimental period. Other components increased initially and then decreased during the remainder of the period. These cylical variations may be attributed to changes in anobolic-catobolic balance.

Smith, A. C. 1976. Catalase in fish red blood cells. Comp. Biochem. Physiol. 54B:331-332.

Catalase activity was measured in the red blood cells of four species of Hawaiian fish (palani, naso, butterfly fish, and milkfish). Correlations were found between taxonomic relationships and variations in catalase activity and red cell changes. Catalase was found in all species, except the milkfish, which was the most primitive species examined.

Smith, C. G., W. W. Lewis, and H. M. Kaplan. 1952. A comparative morphologic and physiologic study of fish blood. Prog. Fish-Cult. 14:169-172.

The morphology and physiology of blood sampled from 10 species of freshwater fish were compared. Interspecific comparisons were made on erythrocyte counts, hemoglobin content, blood coagulation time, mean erythrocyte hemoglobin, and mean erythrocyte cellular and nuclear size. There were pronounced interspecific differences in erythrocyte count, hemoglobin content, blood coagulation time and erythrocyte cellular and nuclear size. An intraspecific inverse correlation was found between erythrocyte size and red blood cell counts.

Smith, L. S. and G. R. Bell. 1964. A technique for prolonged blood sampling in free-swimming salmon. J. Fish Res. Bd. Can. 21(4):711-717.

A technique for cannulation of the dorsal aorta was described for freeswimming, confined salmon. This would permit serial blood sampling or vascular injection and may be used to estimate blood volumes.

Snedecor, G. W. and W. G. Cochran (1968). Statistical Methods. 6th ed. The Iowa State University Press, Ames, Iowa. 593 pp.

Snieszko, S. F. 1961. Microhematocrit values in rainbow trout, brown trout and brook trout. Prog. Fish-Cult. 23(3):114-119.

Hematocrit values were measured in hatchery-reared rainbow trout, brown trout, and brook trout. Similar values were found for brook and rainbow trout. Hematocrit levels were lower in brown trout than in rainbow or brook trout. Significant differences were found between the sexes of sexually mature rainbow, brown, and brook trout.

Spaargaren, D. H. 1976. A comparative study on the regulation of osmotic, ionic and organic-solute concentrations in the blood of aquatic organisms. Comp. Biochem. Physiol. 53A:31-40.

Data on osmotic, ionic, and organic solute regulations in the blood of teleosts and crustaceans were fitted into a third-power polymonial function formula. Some characteristics of the regulation patterns were defined.

Spieler, R. E. 1974. Short-term serum cortisol concentrations in goldfish (*Carassius auratus*) subjected to serial sampling and restraint. *J. Fish. Res. Bd. Can.* 11(7):1240-1242.

> No significant variations in cortisol levels were found in serially sampled goldfish until 10-22 minutes after initial capture. No diurnal variations were found in the response of serum cortisol to handling and restraint stress.

Spieler, R. E. and A. H. Meier. 1976. Short-term serum prolactin concentrations in goldfish (*Carassius auratus*) subjected to serial sampling and restraint. *J. Fish. Res. Bd. Can.* 33(1):183-186.

> The effects of serial sampling and restraint on the serum prolactin levels was investigated in goldfish. Serum prolactin levels decreased approximately 12 minutes after initial capture. These values returned to normal values in approximately 40 minutes after initial capture. Although serum prolactin levels varied diurnally, no circadian variations were found in the response of serum prolactin to handling and restraint stress.

Spoor, W. A. (1951) Temperature and the erythrocyte count of the goldfish. *Am. Physiol. Soc.* 10(1):131.

Stanley, J. G. and P. J. Colby. 1971. Effects of temperature on electrolyte balance and osmoregulation in the Alewife (*Alosa pseudoharengus*) in fresh and seawater. *Trans. Amer. Fish. Soc.* 4:621-638.

> The effects of temperature and salinity on ionic and osmotic regulation were examined in the alewife (*Alosa pseudoharengus*). Abrupt cold exposure resulted in decreased sodium and calcium levels in freshwater fish and increased levels in seawater fish. Exposure to elevated temperatures in freshwater had no effect on plasma electrolyte concentration, whereas in seawater the sodium, potassium, and calcium concentrations were increased at high temperatures. Normal blood electrolyte concentrations were found in alewives thermally acclimated in the laboratory and in fish from Lake Michigan.
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> Muscle sodium concentrations decreased during abrupt cold exposure in both freshwater and seawater. The effects of heat exposure on muscle electrolyte concentrations were generally opposite to those of acute cold exposure. The data suggested that water or ions may move between the cellular and extracellular fluids of the muscles. Exposure to temperature changes resulted in similar mortality levels between alewives held in freshwater and those held in seawater.

Summerfelt, R. C., W. M. Lewis, and M. G. Ulrich. 1967. Measurement of some hematological characteristics of the goldfish. *Prog. Fish-Cult.* 29(1):13-20.

Baseline hematological studies were done on goldfish. Hematocrit and hemoglobin levels were higher in males than in females. No significant differences in serum protein levels, erythrocyte fragility or blood coagulation time were found between males and females. Significant correlations were found between hematocrit and hemoglobin levels.

Toews, D. P. and C. P. Hickman, Jr. 1969. The effect of cycling temperatures on electrolyte balance in skeletal muscle and plasma of rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* 20:905-918.

The effect of diurnal temperature cycles (ranging from 8° to 18°C) on electrolyte balance in muscle and plasma of *Salmo gairdneri* was investigated. Control fish were held at constant temperatures ranging from 8° to 18°C. There were no significant differences in plasma water content in both the cycling fish and the control fish at any temperature. Plasma chloride levels in the cycling fish, which increased between 8° and 14°C on the increasing phase of the cycle and then decreased for the remainder of the cycle, were not significantly different from those of the control fish held at 8° to 10°C. Plasma sodium and potassium values in cycling fish were also similar to values of control fish held at 8° and 10°C.

Muscle chloride levels were relatively stable in control and cycled fish. There was an inverse relationship between muscle sodium and potassium in cycled fish, which was not present in control fish. The data from this experiment suggested that cycled trout apparently acclimated to the lowest temperature of the cycle.

Umminger, B. L. 1969a. Physiological studies on supercooled killifish (*Fundulus heteroclitus*). I. Serum inorganic constituents in relation to osmotic and ionic regulation at subzero temperatures. *J. Exp. Zool.* 172:283-302.

When male *Fundulus heteroclitus* were acclimated to various temperatures (30°, 20°, 10°, 4°, 2°, -1°, and -1.5°C) in seawater, there were physiological and chemical changes in the blood and tissues. The pH of the blood was highest at 10°C. Hematocrit values were significantly reduced when water temperature was decreased from 20°C to 10°C, but did not change with further temperature reduction. When temperatures were reduced from 20°C to -1.5°C, the total serum osmolarity increased, while the percentage of osmolarity attributed to serum inorganic electrolytes decreased. In fish subjected to subzero cold there were also increases in serum sodium, calcium, chloride, magnesium, and bicarbonate concentration, but there were no changes in serum potassium and inorganic phosphate levels. In addition, the water content of the liver, testes, and muscle decreased in subzero temperatures. Although osmotic and ionic regulation were not as effective in the subzero cold, there did not appear to be any indications of osmoregulatory failure resulting in death.

Umminger, B. L. 1969b. Physiological studies on supercooled killifish (Fundulus heteroclitus). II. Serum organic constituents and the problem and supercooling. J. Exp. Zool. 172:409-424.

To aid in examination of survival of seawater-adapted killifish at subzero temperatures, previous studies on serum inorganic constituents were complimented with studies on organic components in parallel groups of male killifish, which were acclimated to temperature ranging from -1.5°C to 30°C. Low temperature had little or no effect on serum total nonprotein nitrogen, total amino acids, urea, and nonglucose free carbohydrate levels. Total serum protein concentrations were usually constant in the cold, but declined occasionally. Cholesterol values were significantly elevated in subzero cold. Serum glucose levels of supercooled killifish were markedly elevated, while serum inorganic and organic components changed little. Hyperglycemia was discussed with regard to survival of fish at subzero temperatures.

Umminger, B. L. 1970a. Physiological Studies on supercooled killifish (Fundulus heteroclitus). III. Carbohydrate metabolism and survival at subzero temperatures. J. Exp. Zool. 173:159-174.

When Fundulus heteroclitus were exposed to subzero cold, there was an immediate increase in serum glucose, a decrease in hepatic glycogen and occasionally a decrease in muscle glygocegn. During prolonged exposure to subzero cold, hepatic glycogen progressively declined, approaching depletion in 15-25 days. Although serum glucose values remained elevated during long periods of exposure to -1.5°C, there was an eventual decrease in glucose levels to normal values, followed by death. Serum nonglucose free carbohydrate and liver protein levels were not affected by exposure to subzero cold, while muscle protein levels decreased only after prolonged exposure to -1.5°C.

When glucose was added to the water of killifish exposed to subzero cold, serum glucose levels and fish survival time were increased. Winter fish, which had higher serum glucose levels and lower gonosomatic index levels, lived longer than summer fish at subzero temperatures.

Umminger, B. L. 1970b. Osmoregulation by the killifish, Fundulus heteroclitus in fresh water at temperatures near freezing. Nature 225:294-295.

Serum chemistry was examined in male Fundulus heteroclitus acclimated to 11°, 4°, or 0.1°C. There were significant decreases in the concentrations of serum sodium and chloride when killifish were exposed to low temperature. The serum osmolarity also decreased on exposure to 0.1°C, but not as much as the combined concentrations of sodium and chloride. In killifish exposed to cold, there appeared to be an increase in unknown serum constituents which lessened the reduction of serum osmolarity.

Umminger, B. L. 1970c. Effects of temperature on serum protein components in the killifish, *Fundulus heteroclitus*. *J. Fish. Res. Bd. Can.* 27(4):404-409.

> Serum protein components were examined in killifish acclimated to temperatures ranging from 1.5° to 30°C. Electrophoresis revealed that 8 distinct zones, which were grouped in zones I, II, and III, were present at all temperatures. In cold-acclimated killifish, there was a greater decrease in low mobility proteins (zone III) than in high mobility proteins (zones I and II). The possible relationship between low mobility proteins and the immune responses in cold-acclimated killifish was discussed.

Umminger, B. L. 1971. Osmoregulatory role of serum glucose in freshwater-adapted killifish (*Fundulus heteroclitus*) at temperatures near freezing. *Comp. Biochem. Physiol.* 38A:141-145.

> Freshwater-adapted *Fundulus heteroclitus* were acclimated to 11, 4°, and 0.1°C. Cold acclimation resulted in decreased serum sodium (30%), chloride (42%), and osmolarity (15%) values, and increased serum glucose (1,976%) values. The data suggested that hyperglycemia helped to maintain serum osmolarity values in cold-acclimated killifish.

Vaccaro, A. M., R. Raschett, G. Ricciardi, and G. Morpurgo. 1975. Temperature adaptation at the hemoglobin level in *Carassius auratus*. *Comp. Biochem. Physiol.* 52A:627-634.

> The effects of temperature (4°, 21°, 37°C) on hemoglobin levels was studied in goldfish. Goldfish possessed a single molecular form of hemoglobin which was not altered during temperature acclimation. Temperature acclimation resulted in significant fluctuations in the oxygen dissociation curves of the hemoglobin. The oxygen affinity of the hemoglobin remained constant during acclimation.
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> The cofactor, which was different from organic phosphates, appeared to minimize temperature effects on the oxygenation reaction and to control the size of the Bohr effect.

Watson, L. J., I. L. Schechmeister and L. L. Jackson. 1963. The hematology of goldfish, *Carassius auratus*. *Cytologia* 28:118-130.

> Blood cell composition and morphology was examined in peripheral blood samples from goldfish held at 22°-24°C for two weeks. Erythrocytes were the most abundant cell type found. Thrombocytes were the second most abundant cell type found. The leucocytes consisted of 92.5% lymphocytes, 5.1% neutrophils, 2.2% eosinophils, and 0.2% basophils. No monocytes were found. Morphology of the cell types were discussed.

Watson, M. E., R. W. Guenther, and R. D. Royce. 1956. Hematology of healthy and virus-diseased sockeye salmon, *Oncorhynchus nerka*. *Zoologian: New York Zoological Society* 41(5):27-41.

The hematology of healthy and virus-diseased fingerling sockeye salmon was examined. In naturally infected and experimentally infected fish, peripheral blood samples were examined at intervals throughout all stages of the virus disease. Normal blood cell types present in salmon consisted of erythrocytes, lymphocytes, thrombocytes, and neutrophils, while eosinophils, basophils, and macrophages were uncommon in healthy fish. The mean hematocrit value for healthy salmon was 47%. Changes in hematocrit and blood cell morphology were associated with viral disease in fish.

Weber, R. E., S. C. Wood, and J. P. Lomholt. 1976. Temperature acclimation and oxygenbinding properties of blood and multiple hemoglobins of rainbow trout. *J. Exp. Biol.* 65:333-345.

Acclimation of rainbow trout to 5° and 15°C for 4 weeks did not appear to significantly alter hematocrit, nucleoside triphosphate, hemoglobin, or nucleoside triphosphate/hemoglobin ratio levels in the blood. The nucleoside triphosphate and nucleoside triphosphate/hemoglobin ratios did not appear to change significantly with the acclimation of trout to 22°C for 12 weeks. However, significantly higher hematocrit and hemoglobin levels were found for rainbow trout acclimated to 22°C for 12 weeks than those for trout acclimated to 5°C for 12 weeks. Thin layer chromatography studies revealed that ATP was the main nucleoside triphosphate present in rainbow trout blood and that guanosine triphosphate constituted less than 10% of the nucleoside triphosphate present in trout blood. When rainbow trout were acclimated to 5, 15, and 22°C for periods in excess of 12 weeks, there appeared to be no significant effect on the oxygen affinity of whole blood. However, slight differences were observed in the oxygenation properties of stripped hemolysates of warm- and cold-acclimated trout. These differences appeared to be correlated with variations in the relative concentrations of multiple hemoglobins. The differences found in the hemolysates may be attributed to the oxygen-binding properties of the major hemoglobin components. The possible thermoacclimatory role of variations in multiple hemoglobin and cofactor concentrations was discussed.

Wedemeyer, G. A. 1976. Physiological response of juvenile coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*Salmo gairdneri*) to handling and crowding stress in intensive fish culture. *J. Fish. Res. Bd. Can.* 33(12):2699-2702.

The effects of crowding and handling on blood glucose levels were examined in coho salmon and rainbow trout. Fish were transferred from a loading density of 0.5 lb/ft^3 to 1, 2, 4, 6, or 12 lb/ft^3. Significant increases in blood glucose were found in rainbow trout moved to densities greater than or equal to 1 lb/ft^3. The glucose levels at all densities returned to normal within 12 days.

Hyperglycemia was found in juvenile coho salmon moved to densities of 6 and 12 lb/ft^3. Although there appeared to be some adaptation to the higher densities, blood glucose levels of the 12 lb/ft^3 group were still somewhat elevated after 16 days. Transfer of smolting coho salmon to 1, 2, 4, 6, or 12 lb/ft^3 resulted in hyperglycemia, which was more severe in densities greater than or equal to 2 lb/ft^3.

The effect of handling and crowding stress on feeding behavior was also discussed.

Williams, R. J. and M. C. Warner. 1976. Some observations on the stained blood cellular elements of channel catfish, *Ictalurus punctatus*. *J. Fish. Biol.* 9:491-497.

Blood was collected from channel catfish periodically during February to August. The blood cell types observed in this species were thrombocytes, lymphocytes, monocytes, neutrophils, eosinophils, basophils, granular anucleate bodies and erythrocytes. Erythrocytes were found in various stages of development. The most predominant leucocytes were thrombocytes, lymphocytes, and neutrophils. Descriptions and sizes of the different cell types were given.

Wilson, R. P. and W. E. Poe. 1974. Nitrogen metabolism in channel catfish, *Ictalurus punctatus*. III. Relative pool sizes of free amino acids and related compounds in various tissues of the catfish.

The tissue and serum contents of free amino acids and related compounds were determined in pond-cultured channel catfish subjected to starvation for 2 days. The major compounds present in all of the tissues and serum were taurine and ammonia.

Liver and kidney generally had the highest concentrations of amino acids and related compounds. However, muscle tissue had the largest percentage of most compounds. The major protein amino acids found were alanine, glycine and glutamic acid.

Yoffey, J. M. 1929. A contribution to the study of the comparative histology and physiology of the spleen, with reference chiefly to its cellular components. I. In fishes. *J. Anat.* 63:314-344.

Young, R. T. 1949. Variations in the blood cell volume of individual fish. *Copeia* 3:213-218.

Hematocrit values were monitored in individual opaleye (*Girella nigricans*) periodically. No correlation was found between weight, or temperature and blood cell volumes. Except for extreme cases of anemia, no relationship was found between hematocrit and feeding responses. There was no diurnal variation in hematocrit levels. There appeared to be no significant differences between hematocrit levels of males and females. Variations in hematocrit levels of individual fish were similar to those found in different fishes.